

INFLUENCE OF MAILLARD REACTION PRODUCTS ON LIPID OXIDATION  
IN A COOKED MEAT MODEL SYSTEM

by

MOHAMMED A. ALFAWAZ

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Approved by:

*William G. Skins*

Major Professor

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## INTRODUCTION

Lipid oxidation is a common occurrence in foods, and it is one of the major causes of quality deterioration in foods such as uncured, precooked, and cooked meat products. Development of oxidative rancidity in meat products limits the shelf life and contributes to the reduction of quality characteristics such as color, flavor, texture, nutritive value, and safety (Pearson et al., 1983). Thus, control of lipid oxidation in meat and meat products is extremely important for economic, nutritional, and food safety reasons.

The term warmed-over flavor (WOF) was first coined by Tims and Watts (1958). These authors employed this term to describe the rapid onset of oxidative rancidity in cooked meats during refrigerated storage. WOF becomes detectable within 48 hours in cooked meats as opposed to the relatively slow onset of rancidity commonly encountered in raw meats. Rancidity in uncooked meat becomes apparent only after several weeks or months of cold storage (Pearson and Gray, 1983). WOF has also been detected in raw meat that has been ground (Greene, 1969; Sato and Hegarty, 1971; Benedict et al., 1975). In general, any process that disrupts muscle cell membranes and thus increases their exposure to oxygen enhances the development of WOF (Gray and Pearson, 1987). Lipid oxidation in cooked meat is thought to be due to the oxidation of phospholipids (Igene and Pearson, 1979; Igene et al., 1981). The catalyst may be nonheme iron which is released from myoglobin and hemoglobin (Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979) by the action of hydrogen peroxide ( $H_2O_2$ ) (Rhee et al., 1987), heating (Schricker and

Miller, 1983; Rhee and Ziprin, 1987) or grinding (Sato and Hegarty, 1971; Pearson et al., 1983a).  $H_2O_2$  has also been shown to react with metmyoglobin and rapidly generate active species which initiate membranous lipid oxidation (Kanner and Harel, 1985; Harel and Kanner, 1985 & 1985a; Rhee et al., 1987). This problem has recently become an important issue due to the increasing demand for precooked restructured meat dishes in the marketplace, restaurant, fast food service, airlines, and institutional service.

Restructuring of meat is a process which requires a particle size reduction or modification, blending and forming of lower value, less utilized and less desirable fresh meat cuts into more convenient, palatable products such as beef steaks and roasts (Breidenstein, 1982; Chastain et al., 1982). The advantage of restructured products over intact muscle meat is that it is possible to produce a product that is consistent in quality, composition (i.e. fat content), size and price (Breidenstein, 1982). Since restructured products are subjected to processes such as grinding and precooking, the integrity of muscle cell membranes is disrupted and the predominantly unsaturated lipids of the membranes are exposed to oxygen and catalysts (Pearson and Gray, 1983; Gray and Pearson, 1987). Therefore, precooked restructured meat products are particularly susceptible to WOF. In addition to the exposure to oxygen and disruption of cell membranes, precooked restructured meat products usually have the added salt which may act as prooxidant (Watts, 1962; Lamkey et al., 1986). This development of WOF in precooked meats is a major obstacle to the acceptability of precooked restructured meat products by consumers (Miller et al., 1986).

A promising approach to retarding WOF in precooked restructured meat is through the use of natural antioxidant substances. Natural antioxidants are becoming more popular as public mistrust of synthetic antioxidants increases. Some of this apprehension is the result of research reporting toxic effects of high concentrations of compounds like butylated hydroxy toluene (BHT) on experimental animals (Witsthe, 1981). A portion of this perception is also due to the assumption by the consumer that a natural compound is automatically safer than a synthetic one. A possible antioxidant of natural origin is Maillard reaction products (MRP). These compounds are formed from the reaction of reducing sugars and amino acids or peptides. Zipser and Watts (1961) first noted the development of an antioxidant activity in overcooked beef. The antioxidant effect was undoubtedly due to formation of MRP through the interaction of sugars and amino compounds during excessive heating. Sato et al. (1973) demonstrated that retorted meat possessed strong antioxidant activity against the development of WOF. These authors attributed this activity to the formation of MRP during the overheating process.

The objectives of this research were to determine if MRP formed from the reaction of monosaccharide with protein hydrolysate were effective in delaying the development of lipid oxidation in a cooked meat model system containing highly unsaturated membrane lipids. A second objective was to determine which molecular weight fractions of the browning pigments were most effective as antioxidants in this system. Different methods of obtaining the protein hydrolysate were also compared. The final objective was to compare the

antioxidative properties of the browning products produced by spray drying versus those produced by refluxing in the same model system. By identifying these conditions or procedures which produce MRP of optimal antioxidative capability in the cooked meat model system, an antioxidant of natural origin that is suitable for precooked restructured meats may be created.

## LITERATURE REVIEW

Lipids have several important functions in living tissues. They may serve as structural components of membranes, provide energy reserves, function as a protective layer for cells, tissues, and vital organs and/or act as cell-surface components that function in cell recognition, species specificity, and tissue immunity (Lehninger, 1975). However, lipid oxidation is a major cause of deterioration in the quality of muscle foods. This quality deterioration involves the development of off-aroma and flavors from the formation or modification of volatile odor compounds such as aldehydes. The off-taste can be caused by the formation of hydroxy acids. The color can be altered by a Maillard type of reaction between reducing substances originating from lipids and proteins. The texture can be affected by cross-linking reactions of proteins. In addition, the nutritive value can be decreased due to the loss of essential fatty acids. The safety of food can also be jeopardized with the formation of food toxins such as cholesterol oxides (Eriksson, 1987).

### A. Autoxidation

Autoxidation of food lipids is promoted by heat, light, singlet oxygen, and trace metal catalysts, especially copper and iron (Ingold, 1967; Waters, 1971). The rate and degree of autoxidative deterioration of lipids is directly related to the amount of unsaturation, the concentration of prooxidants and antioxidants and to the cellular and tissue structure (Love and Pearson, 1971). Lundberg (1962) has reviewed the mechanisms involved in autoxidation. The free

radical chain theory of autoxidation has been widely accepted as the mechanism of oxidation in unsaturated fatty acids. Once started, the reaction proceeds by chain reaction and is autocatalytic in that the oxidation products catalyze the reaction and cause an increase in the reaction rate as oxidation proceeds. The steps involved are illustrated in the following simplified scheme.

a. Initiation:



The initiation of the reaction involves the abstraction of hydrogen to form a free radical species in the presence of an initiator. It may take place by hydroperoxide decomposition, metal catalysis, singlet oxygen, or by exposure to light (Fennema, 1985).

b. Propagation:



The addition of oxygen occurs at the carbon atom adjacent to the double bonds (methylene carbon), resulting in the formation of peroxy radicals ( $\text{ROO}\cdot$ ), and these in turn abstract hydrogen from  $\alpha$ -methylene groups ( $\text{RH}$ ) of the other fatty acid molecules to yield hydroperoxides ( $\text{ROOH}$ ) and another free radicals ( $\text{R}\cdot$ ). The new free radicals react with oxygen and the sequence of reactions is repeated. In addition to the formation of hydroperoxides, other types of reactions may occur. The peroxides may decompose to carbonyls, form polymers, or react with proteins, vitamins, pigments, etc.



(Gray, 1978; Karel, 1973). The hydroperoxides are intermediates in the oxidation process. They do not themselves contribute to rancid odors but are unstable. They break down to a variety of products such as aldehydes, ketones, acids and alcohols which contribute to rancid flavors (Frankel, 1984).

c. Termination:



These reactions lead to the formation of inactive stable end products.

**B. Formation of Malonaldehyde**

Malonaldehyde (MA) is a three-carbon dialdehyde which is produced during autoxidation of polyunsaturated fatty acids (Patton and Kurtz, 1955; Kwon and Olcott, 1966 & 1966a). The formation of MA is the basis for the well-known 2-thiobarbituric acid (TBA) method used for measuring the extent of oxidative deterioration of lipid in muscle foods (Gray, 1978; Rhee, 1978). The MA produced from polyunsaturated fatty acids condenses with two moles of TBA to form a highly colored product which is measured spectrophotometrically (Figure 1). Pryor et al. (1976) proposed a modification of the mechanism of Dahle et al. (1962) to more adequately explain why oxidized polyunsaturated fatty acids produce much more MA than fatty acids with one or two double bonds. They suggested that cyclic peroxides, whose formation is shown in Figure 2, produce endoperoxide radicals with five membered rings. These

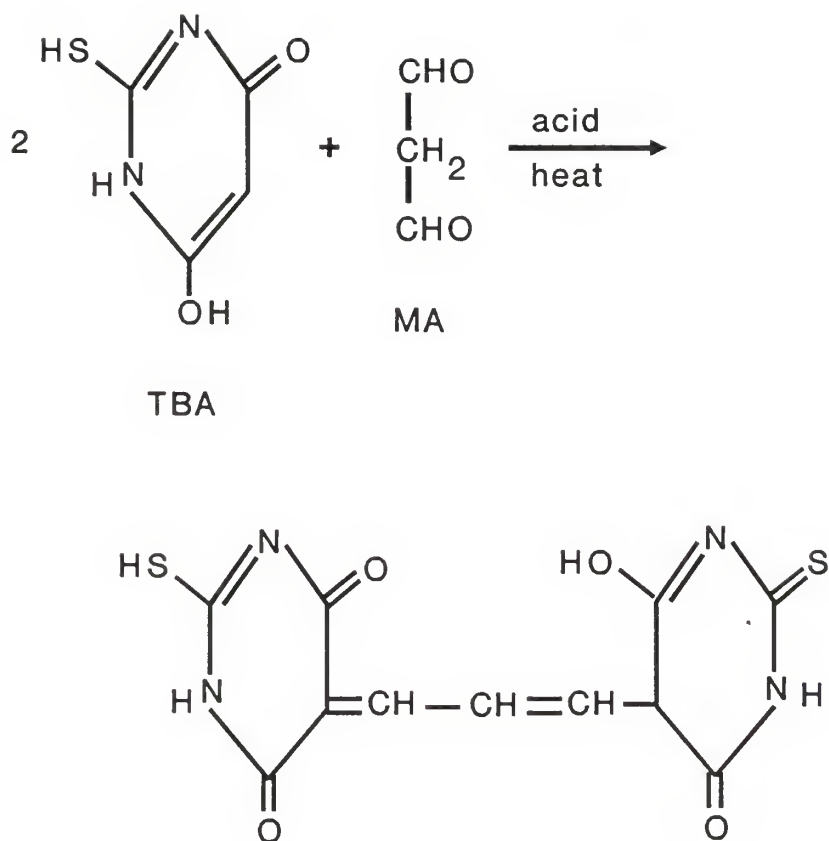


Figure 1. The reaction of TBA with MA (Pryor et al., 1976)



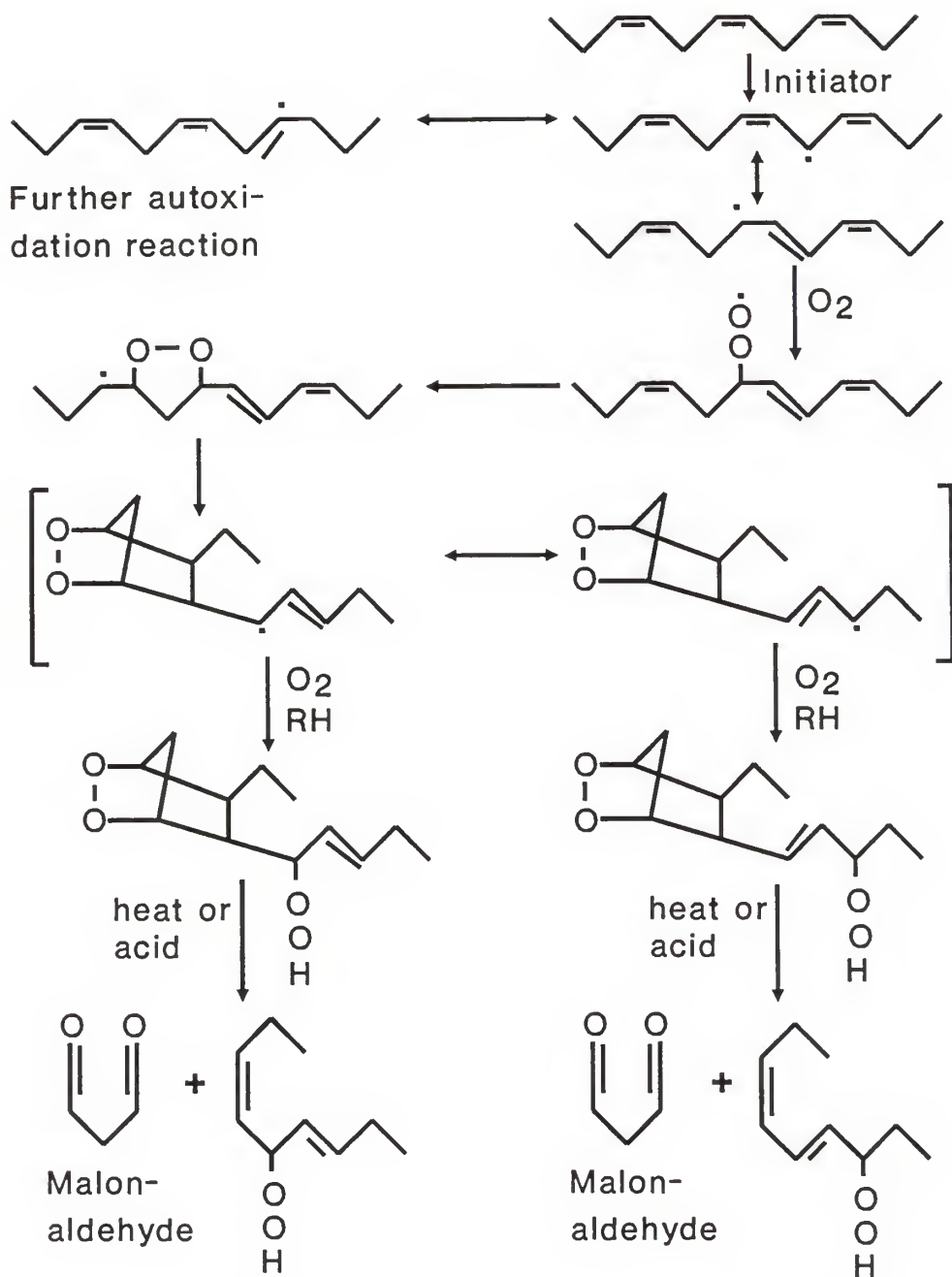


Figure 2. Formation of Malonaldehyde (Pryor et al., 1976)

endoperoxide radicals undergo peroxidation and abstraction of an alkyl hydrogen to produce new compounds. When these compounds are subjected to heat or acid, MA is formed.

### C. Lipid Oxidation in Muscle Foods

In muscle foods, susceptibility to oxidative deterioration has been related to the presence of various prooxidants and antioxidants as well as to the nature, proportion and degree of unsaturated fatty acids in the lipids of these foods. Although animal lipids are considered to be fairly saturated, sufficient amounts of unsaturated fatty acids are found in the phospholipid fraction of the intramuscular lipids. In chicken, beef and lamb, the neutral or nonpolar lipids contain 40-50% monounsaturated fatty acids and less than 2% of the highly unsaturated fatty acids (Allen and Foegeding, 1981). In beef phospholipids 23% of the fatty acids contain three or more double bonds, while only 1.0% of the triglyceride fatty acids show the same degree of unsaturation (Love and Pearson, 1971).

Studies of individual phospholipids have demonstrated that phosphatidyl ethanolamine was the major phospholipid involved in oxidative rancidity in cooked meat (Pearson et al., 1977; Igene and Pearson, 1979; Pearson and Gray, 1983). Substantial evidence has accumulated to support the theory that phospholipids are major contributors to oxidative rancidity in cooked beef, lamb and sea foods with triglycerides playing only a minor role (Igene et al., 1980; Melton, 1983; Wilson et al., 1976).

#### D. Warmed-Over Flavor

The term warmed-over flavor (WOF) was first recognized by Tims and Watts in 1958. It is a form of oxidative rancidity that develops rapidly in cooked meat during refrigerated storage. This rancid or stale flavor becomes readily apparent within 48 hours in contrast to frozen stored raw meat that requires months to develop rancidity (Pearson et al., 1977; Igene et al., 1979a, 1980). It also develops in raw meat in which the membranes are disrupted by processes such as restructuring or grinding (Gray and Pearson, 1987). It appears that very little time is required for the initiation of off-flavor development. This problem has recently become a major issue in the consumer acceptance of precooked meats such as restructured products (Miller et al., 1986).

WOF is primarily due to the oxidation of the highly unsaturated membrane phospholipids, especially phosphatidyl ethanolamine (Igene and Pearson, 1979; Willemot et al., 1985). Cooking denatures meat pigments and results in an increase in the amount of nonheme iron (Igene et al., 1979). Cooking also disrupts meat tissues, and thus increases the release of polyunsaturated fatty acids in cooked meat (Igene et al., 1981). Therefore, cooking permits intimate mixing of lipid substrates and catalysts and results in an increased rate of lipid oxidation.

Greene et al. (1971) and Love and Pearson (1971) reported that myoglobin served as a prooxidant in meat. However, Sato and Hegarty (1971), Love and Pearson (1974) and Igene et al. (1979) demonstrated that nonheme iron, not myoglobin, catalyzed lipid oxidation in model meat system. Love and

Pearson (1974) treated a water-extracted meat system with metmyoglobin at levels of 1-10 mg/g of muscle. This treatment had little effect on oxidation, while the addition of 1 ppm of ferrous iron ( $\text{Fe}^{+2}$ ) increased lipid oxidation dramatically. Tichivangana and Morrissey (1985) confirmed this study using the same model system.

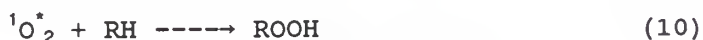
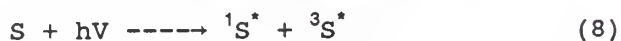
Liu and Watts (1970) suggested that both heme and nonheme iron may be responsible for the catalysis of lipid oxidation. Their suggestion was based on the observation that lipid oxidation still occurred in cooked meat samples whose heme pigments were destroyed by treatment with 30%  $\text{H}_2\text{O}_2$  and that the oxidation of lipids also occurred in untreated cooked meat.

## E. Catalysis of Lipid Oxidation

### 1. Singlet Oxygen

Oxygen molecules exist in its ground or triplet state, which is unusual, since most molecules have a singlet ground state (Chan, 1987). The oxygen molecule in its triplet state contains two unpaired electrons with parallel spin but in different orbitals. These electrons are kept apart and, therefore, have only a small repulsive electrostatic energy. Unsaturated fatty acids in the singlet state contain no unpaired electrons. It is, therefore, thermodynamically difficult for oxygen to react with fatty acids. This means that there is a spin barrier that prevents the direct addition of triplet oxygen to the singlet-state of unsaturated fatty acids. In the singlet oxygen state, the two electrons have opposite spins, and thus, electrostatic repulsion will be great. Therefore, singlet oxygen reacts readily with unsaturated fatty acids.

Singlet oxygen can be formed by a reaction involving the presence of light and a sensitizer (Rawls and Van Santen, 1970). It is important to distinguish between this sensitized photooxidation and photolytic autoxidation, which can be initiated by UV-catalyzed decomposition of hydroperoxides (Frankel, 1984). Potential sensitizers in meat are the hemoproteins, myoglobin and hemoglobin. The mechanism involves the activation of a sensitizer (S) by light to form an excited sensitizer ( $^3S^*$ ). The excited sensitizer reacts with triplet oxygen ( $^3O_2$ ) to form excited singlet oxygen ( $^1O_2$ ) which can react directly with unsaturated fatty acids to produce hydroperoxides. The hydroperoxides decompose to free radicals which enter the chain reaction of lipid oxidation. This process is summarized in the following reactions.



Singlet oxygen can be inhibited by quenchers such as  $\beta$ -carotene and  $\alpha$ -tocopherol that deactivate  $^1O_2$  to the triplet state (Foote, 1976).

The formation of hydroperoxides by singlet oxygen proceeds by a mechanism different than that of free radical autoxidation. Singlet oxygen reacts directly with the double bonds by addition in a so called "ene" reaction. Oxygen is thus inserted at one end of the double bond which shifts to yield allylic hydroperoxides. This would give 9- and 10-hydroperoxides for oleic acid instead of the 8-, 9-, 10- and 11- hydroperoxides of the free radical autoxidation.

## **2. Heme Pigments and Nonheme Iron**

The catalytic effects of heme iron and nonheme iron on lipid oxidation in meat systems have been implicated as the major prooxidants in meat and meat products (Younathan and Watts, 1959; Liu and Watts, 1970; Greene et al., 1971). Myoglobin was initially viewed as the major catalyst of lipid oxidation (Tappel, 1962). However, studies by Sato and Hegarty (1971) and further development by Love (1972) and Igene (1978) revealed that nonheme iron, rather than heme iron, was the active catalyst responsible for the rapid oxidation of cooked meat. The majority of the studies in the 1960's and 1970's dealt with the catalytic decomposition of preformed lipid hydroperoxides. Ferrous iron is known to decompose lipid hydroperoxides, forming very reactive alkoxy radicals for the propagation reactions (Ingold, 1962). Similarly, the mechanism proposed by Tappel (1962) depends on the presence of lipid hydroperoxides which react with heme compounds and undergo homolytic decomposition. Heme proteins are thus catalyzers of the propagation step and not truly initiators of lipid oxidation. The ability of heme pigments and nonheme iron to accelerate the propagation step of the free radical chain mechanism can explain the rapid rate of lipid oxidation in cooked meats (Igene et al., 1979; Tichivangana and Morrissy, 1985). In a recent review by Love (1983), the role of heme and nonheme iron in promoting lipid oxidation in muscle foods was fully discussed. It appears that hemoproteins are a source of nonheme iron and during the cooking process, the nonheme iron is released and is the major catalyst for lipid oxidation in cooked meats.



### 3. Hydrogen Peroxide

Hydrogen peroxide and the superoxide radical are normally present at low concentration as metabolites in aerobic cells (Ramasarma, 1982). Microsomes (Hildebrandt and Roots, 1975), mitochondria (Boveris et al., 1972) and peroxisomes (Boveris et al., 1972) have been recognized as effective  $H_2O_2$  generators when fully supplemented by their substrates.

Hydrogen peroxide and the superoxide radical are cytotoxic to biological tissues. These intermediates of oxygen have the capacity to generate other reactive oxygen species such as hydroxyl radical that can then initiate a radical chain reaction (Fridovich, 1983).

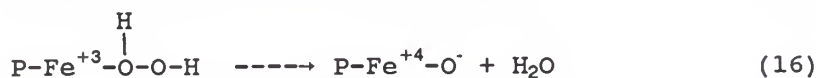
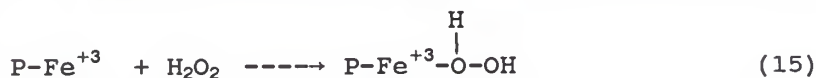
The formation of hydrogen peroxide by muscle tissues could be generated by the activity of enzymes located in mitochondria, peroxisomes as well as by cytosolic enzymes (Boveris et al., 1972; Chance et al., 1979). However, these researchers observed an increased production of  $H_2O_2$  at high temperature. This indicated that the generation of  $H_2O_2$  in muscle tissues can also arise from non-enzymatic reactions. One possible non-enzymatic reaction which could generate  $H_2O_2$  in muscle tissues is the oxidation of oxymyoglobin and oxyhemoglobin. Mirsa and Fridovich (1972) and Satoh and Shikama (1981) reported that the oxidation of oxymyoglobin and oxyhemoglobin led to the formation of superoxide radical and  $H_2O_2$ .

Recently, Kanner and Harel (1985) and Harel and Kanner (1985 & 1985a) have demonstrated that metmyoglobin, when activated by hydrogen peroxide, will initiate membranal lipid oxidation. They proposed that autooxidation of oxygenated heme pigments (oxymyoglobin and oxyhemoglobin) leads to formation

of metheme proteins and superoxide radical ( $\text{O}_2^-$ ), which dismutates to form hydrogen peroxide.

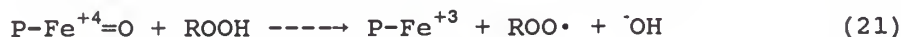
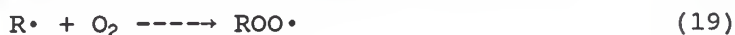


A reactive porphyrin cation radical ( $\text{P}^+-\text{Fe}^{4+}=\text{O}$ ) results from the reaction of metmyoglobin and methemoglobin with hydrogen peroxide.



This radical may then react to produce lipid free radicals which can initiate lipid oxidation in uncooked meat.

The following steps explain the initiation and propagation of membrane lipid oxidation in meat systems according to Kanner and Harel (1985).



Rhee et al. (1987) further studied the mechanism of lipid oxidation in meat systems and concluded that the heme pigment system (MetMb and  $\text{H}_2\text{O}_2$ ) plays a major role in the catalysis of lipid oxidation in raw and cooked meats. They suggested that hydrogen peroxide-activated metmyoglobin was the primary initiator of lipid oxidation in raw meat, and that nonheme iron, released from metmyoglobin by the action of  $\text{H}_2\text{O}_2$ , was the major catalyst of lipid oxidation in cooked meat.



#### **4. Heating**

Igene et al. (1979) have reported that the increase in oxidation activity due to heating is because of the increase in free nonheme iron being released during the cooking process. Schricker et al. (1982), Schricker and Miller (1983) and Chen et al. (1984) have verified the increase in nonheme iron levels in a meat pigment system as a consequence of heating. When meat is heated, denaturation of the protein portion of the molecule might facilitate exposure of iron to unsaturated fatty acids (Love, 1983). Eriksson (1975) showed that protein denaturation increased the ability of the heme-containing proteins to promote lipid oxidation. They proposed that this phenomena was due to the unfolding of the proteins which exposed more heme to the unsaturated fatty acids. This may explain the more rapid lipid oxidation observed in cooked meat. Chen et al. (1984) found that slow heating increased the amount of nonheme iron more than fast heating. The increase in nonheme iron during slow heating appears to be due to the release of heme iron from the porphyrin rings, whereas fast heating may induce coagulation of the myoglobin molecule in such a way that the heme iron could not be cleaved from the globin moiety. Heat could also disrupt the muscle membrane and break lipoprotein complexes which expose unsaturated lipids to oxygen and catalysts and make them more susceptible to oxidative attacks (Dawson and Gartner, 1983).

#### **F. Antioxidants**

A number of antioxidants, both synthetic and natural, have been used to prevent lipid oxidation development in stored meat and meat products. However, there may be two

major problems for the use of synthetic antioxidants. First, consumers are increasingly concerned about chemical additives used in foods. Secondly, synthetic antioxidants are approved for use in only a limited number of meat products and each new synthetic antioxidant must be subjected to a lengthy process of evaluations to insure their safety. Utilization of natural antioxidants of food sources is an alternative to the use of synthetic antioxidants for meat products.

### **(1) Natural Antioxidants**

Many studies on lipid oxidation in meats have focused on the antioxidant activity of naturally occurring substances. These substances include various edible products from vegetable, oilseeds and grains, as well as proteins, amino acids, spices, herbs and protein hydrolysates. Houlihan and Ho (1985) and Rhee (1987) have reviewed the antioxidative nature of these substances. Natural antioxidants may function as reducing compounds, as free radical chain interrupters, as inhibitors of the formation of singlet oxygen, and as chelators of metal ions (Dugan, 1980).

#### **a. Vegetable Extracts**

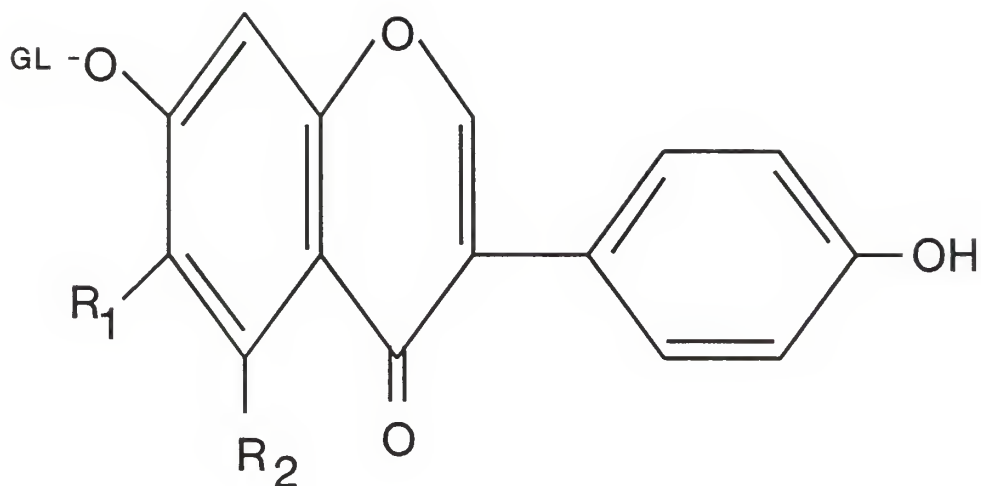
Watts (1961) demonstrated that aqueous extracts of many vegetables, including celery leaves, mint leaves, carrots, white potato skins, beet stems, and mushroom, were effective in retarding lipid oxidation in cooked meats. Watts (1962) found that extracts of onions, potato peelings and green peppers (pods and seeds) were all effective in reducing WOF in sliced roast beef. This protection was found to be related to the flavonoid content of the vegetable extracts. Pratt and

Watts (1964) studied the antioxidant potential of a number of plant extracts and concluded that flavone aglycones were effective antioxidants. Younathan et al. (1980) also showed that ground beef treated with onion juice or textured vegetable protein underwent oxidation at a slower rate than the controls. These authors found that rancidity in turkey was effectively controlled by hot-water extracts of eggplant tissue and peelings of yellow onions, potatoes, and sweet potatoes.

Flavones and flavonoids have molecular structures which make them useful as antioxidants (Figure 3). The relationship between flavone structure and antioxidant activity has been investigated by several workers (Simpson and Uri, 1959; Mehta and Seshadri, 1959; Crawford et al., 1961). The antioxidant activity of these compounds is apparently related to the ketone group in the 4 position of the pyrone ring and 2,3-olefin group. The 3,4 and 5,7-dihydroxy groups apparently contribute to the antioxidant role (Crawford et al., 1961). Pratt et al. (1982) identified three isoflavones and several phenolic acids in soy protein hydrolysates and showed that they contribute to the antioxidant properties of soybeans. Unfortunately, the flavones (e.g., quercetin, as shown in Figure 4) have structural characteristics associated with mutagenic and perhaps carcinogenic activities (McGregor and Jard, 1978).

#### **b. Proteins**

A number of proteins have been shown to retard lipid oxidation. These include milk proteins, especially casein (Taylor and Richardson, 1980), soy proteins (Pratt, 1972) and



Genistein  $R_1 = H$ ,  $R_2 = OH$

Daidzein  $R_1 = H$ ,  $R_2 = H$

Glycitein 7-O-Glucoside,  $R_1 = OCH_3$ ,  $R_2 = H$

Figure 3. Structure of soybean isoflavone glycosides  
(from Pratt and Birac, 1979)

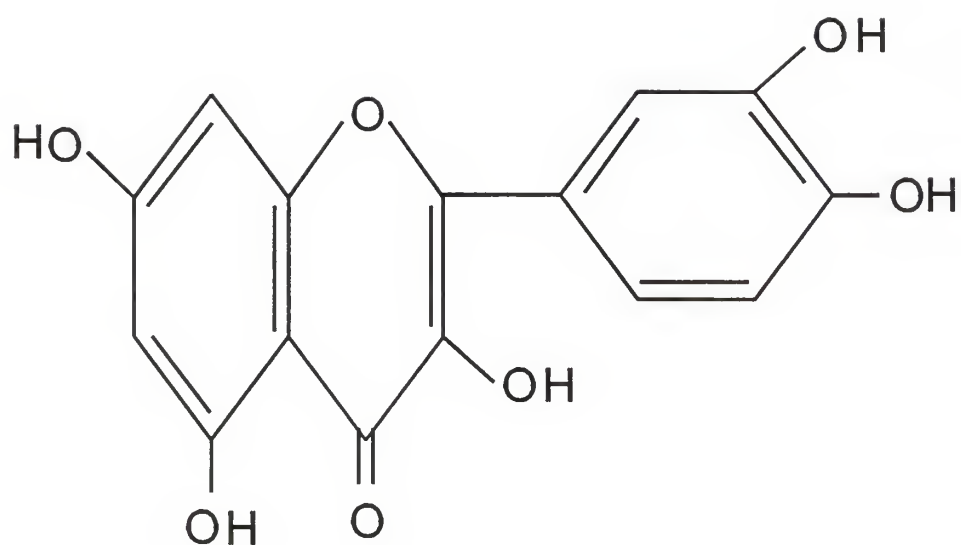


Figure 4. Structure of quercetin (from Rhee, 1987)

serum albumin at low concentration (Yukami, 1972). Bishov and Henick (1975) reported that protein hydrolysates such as hydrolyzed vegetable protein and autolyzed yeast protein possess antioxidative properties. The antioxidant activity of proteins may be due to the amino acid side chains and the atoms involved in the peptide linkages (Bishov et al., 1977).

#### **c. Amino Acids**

Many amino acids have been reported to act as antioxidants (Dugan, 1980). However, the antioxidative effect varies, and they can be prooxidative depending on their concentration and pH. Proline was found to be synergistic (Olcott and Kuta, 1959). Linoleic acid oxidation was shown to be inhibited by cysteine, histidine and alanine at pH 9.5. At pH 7.5, cysteine was strongly prooxidative and both histidine and alanine were antioxidative at low concentration and prooxidative at higher concentration (Marcuse, 1960). Karel et al. (1966) found that histidine, alanine and lysine had an antioxidative effect in a freeze dried system, whereas methionine, arginine, phenylalanine and isoleucine had no effect.

#### **d. Spices and Herbs**

Many spices and herbs have been found to have antioxidative properties. Bishov et al. (1977) studied 19 commercial spices and herbs for their antioxidant effect in freeze-dried model systems, and found that clove, cinnamon, sage, rosemary, mace and nutmeg were highly antioxidative. They also found these spices and herbs to act as strong synergists together with BHA or yeast-protein hydrolysate.

The antioxidant properties of these foods are apparently related to the concentration of phenol like hydroxycinnamic and hydroxybenzoic acids (Eriksson, 1987). Rosemary, for example, contains a number of compounds possessing antioxidant activity, including carnosol, rosmanol, rosmariquinone and rosmaridiphenol. The latter two compounds when tested in lard at a level of 0.02% have been found to be better antioxidants than BHA (Houlihan et al., 1984 & 1985). Barbut et al. (1985) demonstrated that a rosemary oleoresin when added to turkey breakfast sausage at the 20 mg/kg level produced an antioxidative effect comparable to that of a commercial blend of BHA/BHT/citric acid and did not adversely affect overall palatability of the products.

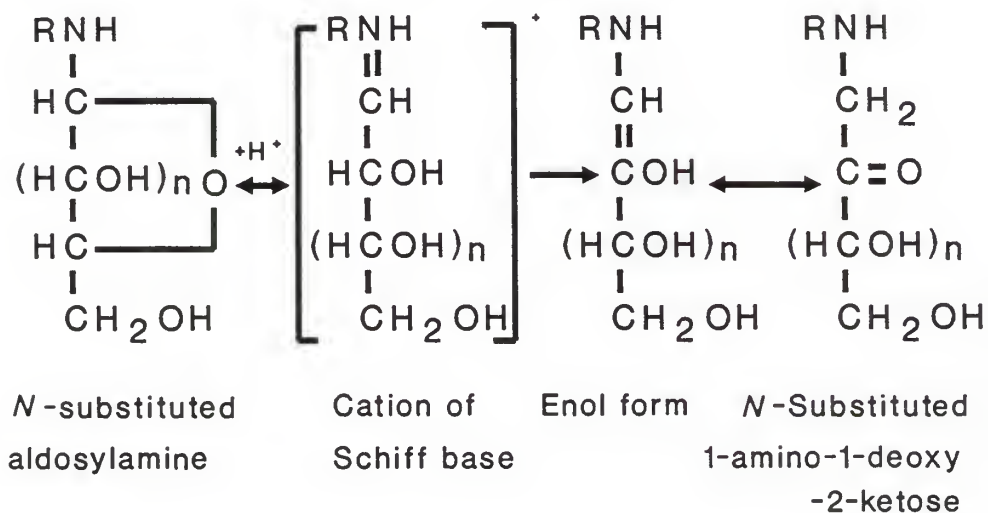
#### **e. Maillard Reaction**

The Maillard reaction is a non-enzymatic chemical reaction involving the interaction of amino groups of amino acids and carbonyl groups of reducing sugars. The reaction is named after the French biochemist Louis-Camille Maillard (1878-1936) who first described the formation of brown pigments or melanoidins upon the heating of a solution of glucose and lysine (Peterson, 1974). The Maillard reaction as a sugar-amine reaction should be distinguished from caramelization which occurs when pure sugars are heated to form brown pigments (Mauron, 1981). The reaction may be desirable as in baked, fried or roasted foods, or undesirable as in concentrated and dried foods. A widely accepted mechanism for the reaction has been published by Hodge (1953), and additional reactions have been suggested by Mauron (1981) and Feather (1981).



The reaction mechanism consists of four major stages (Figure 5). The first is the condensation of the carbonyl group of the reducing carbohydrate and the free amino group of the amino acid or protein. The condensation product rapidly loses a molecule of water and thus is converted into the Schiff base. The Schiff base undergoes cyclization to the corresponding N-substituted glycosylamine. Up to this step, the reaction is reversible. The second phase involves a rearrangement of the glycosylamines in either one of two ways, depending on if the sugar was an aldose or a ketose. Starting with an aldosamine, the product becomes a 1-amino-1-deoxy-2-ketose and is known as the Amadori rearrangement (Figure 5). With a ketosamine, the product becomes a 2-amino-2-deoxyaldose, which is known as the Heynes rearrangement (Reynolds, 1965). These early Maillard reaction products do not cause browning or contribute to overall flavor of the food, although they can reduce nutritive value by reduction of available amino acids (Hurrell and Carpenter, 1974). The third stage involves the loss of the amine from Amadori compound to form carbonyl compounds through at least two distinct pathways (Hodge, 1953). In the first pathway, the Amadori compound forms an enol in positions 2 and 3 irreversibly, eliminating the amine group from C<sub>1</sub> to form a methyl dicarbonyl intermediate (Figure 6) (Hodge, 1953; Hodge and Rist, 1953). These dicarbonyl intermediate further react to give fission products such as C-methyl-aldehydes, keto-aldehydes, dicarbonyls and reductones (Hodge, 1967). The reaction products include such flavor components as acetaldehyde, pyruvaldehyde, hydroxy acetone and acetic acid (Figure 6). The second pathway starts from 1-2-enediol-form





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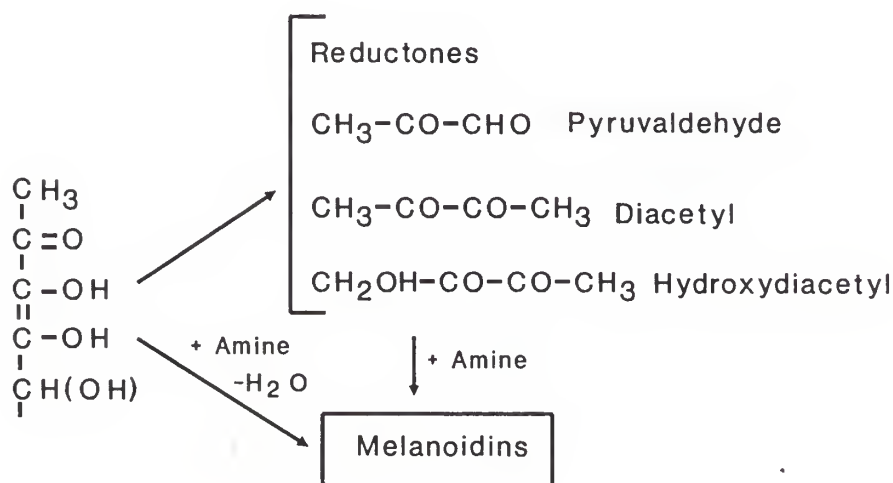
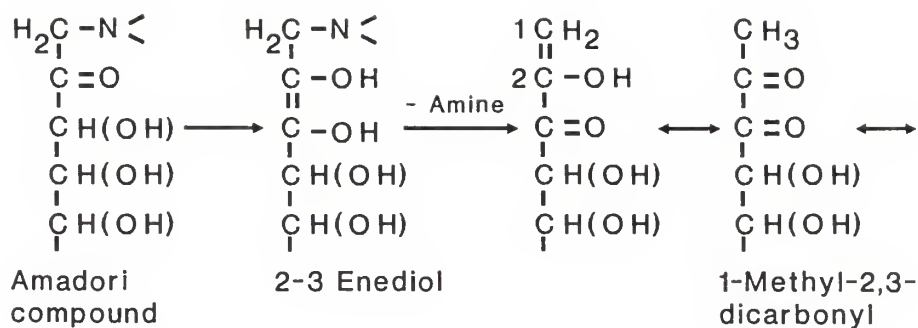


Figure 6. Decomposition pathways of Amadori compound to produce reductones (Hodge, 1967)

of the Amadori product in a reaction that involves the elimination of the hydroxy group at C<sub>3</sub> (Figure 7). The elimination of the amine from C<sub>1</sub> and the addition of water leads to the formation of 3-deoxyhexosone (Anet, 1964). Dehydration then occurs yielding flavor compounds such as maltol, isomaltol and hydroxy methyl furfural (Figure 7). In both pathways, the reactions which follow the formation of these primary intermediates are very complex and only partially understood. The interaction of these intermediates with other constituents during heating causes the formation of N-, O- and S-heterocyclic compounds such as furans, pyrazines, thiazoles, pyrroles, pyridines and polysulfides (Hodge, 1953). Further heating and interaction results in formation of nitrogen- containing pigments known as melanoidins.

#### **1. Antioxidative Properties of the Maillard Reaction Products (MRP)**

The antioxidant activity of MRP is well established. Zipser and Watts (1961) observed an antioxidant effect in over-cooked beef and suggested that diluted slurries could be used to prevent lipid oxidation in normally cooked meat. Bailey et al. (1987) have reviewed the inhibition of warmed-over flavor by MRP.

Hodge and coworkers (Hodge and Rist, 1953; Evans et al., 1958) demonstrated that Maillard browning intermediates have antioxidant activities in lipid systems. These intermediates are resonance-stabilized enediols that react as strong reducing agents. This group of compounds include triose-reductone, dihydroxymaleic acid, reductic acid and dihydroxy

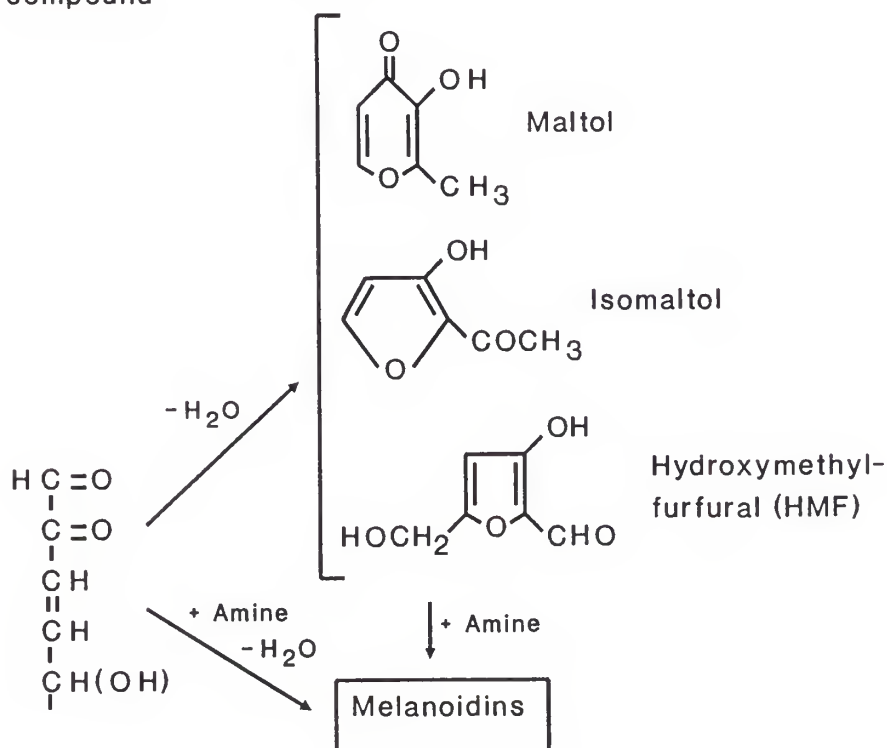
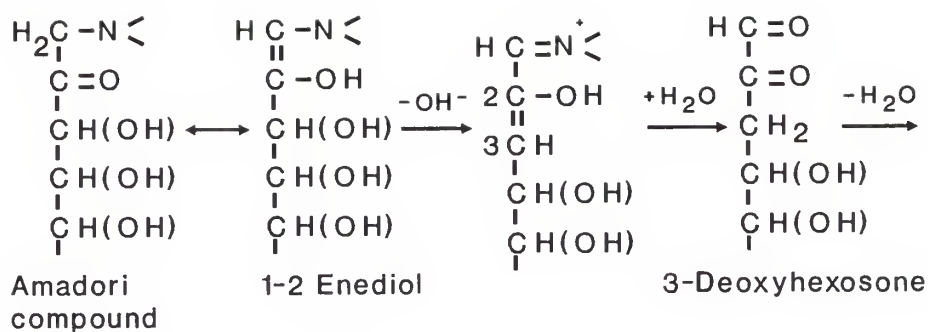


Figure 7. Decomposition pathways of Amadori compound to produce flavor compounds (Hodge, 1967)

pyrogallo. Some of these reductones were later shown to be more effective in preventing oxidation in soybean oil and cottonseed oil than propyl gallate (Mills et al., 1981). Mills et al. (1981) hydrolyzed some of these compounds with 4N HCl and produced and identified a number of highly cyclic reductone-like antioxidants. Their antioxidant capacities as measured by the oxidation of safflower oil were less effective than BHA or BHT but were better than propyl gallate in the presence of a hemoglobin catalyst.

Many attempts have been made to demonstrate the antioxidative potential of MRP using different lipid systems and different methods of measuring antioxidative activity. Most comparisons of activity have been between low molecular weight (dialyzable fractions) and high molecular weight compounds (nondialyzable melanoidins).

Yamaguchi et al. (1981) found differences in activity from different fractions of MRP prepared by heating D-xylose and glycine at 100°C for 2 hr. They separated melanoidins from low molecular weight reductones by Sephadex G-15 gel filtration, and further fractionated the melanoidin fraction on Sephadex G-50 and Sephadex G-100. One fraction of the melanoidins had a molecular weight of 4,500 daltons and exhibited more inhibitory activity on the autoxidation of linoleic acid than BHA, propyl gallate, or erythorbic acid. However, this melanoidin fraction had less antioxidative effect than BHT.

Lingnert and Ericksson (1980) studied the antioxidative activity of MRP produced by refluxing various mixtures of amino acids and sugars using linoleic acid as a model system. Results of the antioxidative effect as measured by a

polarographic method showed that the most antioxidative mixtures contained arginine-xylose and lysine-xylose. Histidine was more effective than valine, cysteine or glutamic acid when heated with the various sugars. They demonstrated that basic amino acids such as arginine, histidine, and lysine, formed strongly antioxidative compounds during reactions with simple sugars. Yamaguchi et al. (1981) also found that melanoidin fractions formed from ammonia and basic amino acids with sugars exhibited better antioxidative activity than other amino acids using linoleic acid as model system. Thus, there may be a proportional relationship between the amount of nitrogen in the melanoidin and its activity as an antioxidant.

Lingnert and Eriksson (1980a) used a model system composed of a linoleic acid emulsion to study the antioxidative effect of MRP produced from sugar with dipeptides or protein hydrolysates as measured by a polarographic and a gas chromatographic method. MRP from dipeptides were formed by refluxing 1 M mole of L-histidylglycine or glycyl-L-histidine hydrochloride with 2 M mole D-xylose in 5 mL 0.1 M potassium phosphate buffer (pH 7) for 5 hr. The antioxidative effect of MRP from dipeptides and sugars was affected by the amino acid sequence of the dipeptide. Thus, greater antioxidative effect was obtained by reacting histidylglycine with xylose than by reacting a mixture of histidine and glycine with xylose. MRP were also obtained by refluxing 1 g of protein hydrolysates of hemoglobin, malt sprouts or brewer's grains with 10 Mmole of D-glucose in 5 ml 0.1 M potassium phosphate buffer (pH 7) for 5 hr. These protein hydrolysates themselves were found to

have some antioxidative effect. However, their antioxidative activities were increased when they were reacted them with glucose.

The influence of pH and the molar ratio of amino acid to sugar on the formation of MRP was studied by Lingnert and Eriksson (1980). They found that neutral or slightly basic pH favored production of antioxidative products. It was demonstrated that at slightly acidic pH, the amino acid group of glycine existed in the ionized form and thus did not exert an antioxidative effect. Furthermore, these authors found that the MRP possessing the greatest antioxidative activity was obtained by refluxing a 2:1 molar ratio of histidine to glucose for 20 hr. However, Beckel and Waller (1983) reported that MRP possessing maximum antioxidative effect was obtained from arginine and xylose mixtures at a molar ratio 1:1 which were refluxed at an initial pH of 5 for 20 hr.

In the application of antioxidative MRP to food, it was found that the addition of histidine and glucose to cookie dough without preforming MRP retarded development of rancidity of cookies (Lingnert, 1980). This treatment was more effective than adding 0.1% preformed MRP from the same reaction mixture. Lingnert and Lundgren (1980) used several experimental procedures to demonstrate that MRP protected sausage from oxidation during frozen storage. Sausages of a frankfurter type were produced from batter containing 0.08% and 0.16% MRP from histidine and glucose, 0.08% histidine and 0.32% glucose, 0.16% histidine and 0.32% glucose and 0.16% MRP from an enzymatic hemoglobin hydrolysate and glucose. The sausages were wrapped separately in aluminum foil and stored at -20°C. During storage, samples were withdrawn for sensory



evaluation, gas chromatographic analysis for volatile compounds and determination of the peroxide value. The sensory evaluation demonstrated that the preformed MRP were capable of retarding lipid oxidation. No effect was obtained from the addition of free histidine and free glucose. Neither the concentration of volatiles, nor the peroxide value supported the results of the sensory evaluation.

## **2. Structure and Mechanism of Antioxidative MRP**

Eichner (1979) indicated that colorless intermediates of MRP such as 1,2-enaminoles derived from Amadori compounds and similar intermediates have powerful antioxidative effects. They are similar in structure to known antioxidants such as ascorbic acid, triose-reductine, dehydroxymaleic acid and reductinic acid. Sato et al. (1973) found that reductic acid and maltol (Figure 8) retard WOF development in cooked ground beef.

There is strong support for the concept that the antioxidative effect of MRP is due to free radicals formed during heating of sugars and amines. Results of electron paramagnetic resonance (EPR) studies of several fractions of a histidine-glucose reaction mixture indicated good agreement between intensity of EPR signal and antioxidative effect (Lingnert et al., 1983). Eichner (1983) observed that the oxygen uptake of a solution containing sodium linoleate and low molecular weight MRP was stopped after some time. He assumed that the MRP reduced hydroperoxides to compounds which are no longer able to form free radicals. Other possible mechanisms include the complexation of heavy metals (Gomyo and Horikoshi, 1976).



## G. Protein Hydrolysates

### 1. Formation of Protein Hydrolysates

Proteins generally consist of up to twenty amino acids joined together through peptide linkages to form a three dimensional structure. The exact sequence of amino acids will be different for each individual protein. When protein is subjected to hydrolysis, the peptide linkages are attacked at many points along the chain and the following fractions can occur during hydrolysis (Prendergast, 1974):

protein ---> primary proteoses ---> secondary proteoses --->  
peptones ---> polypeptides ---> simple peptides ---> amino acids.

Lingnert and Eriksson (1980) suggested the use of protein hydrolysates as the amine source to form MRP. The hydrolysate of hemoglobin was obtained by enzymatic hydrolysis. The red cell fraction of ox-blood was denaturated by adjusting pH to 11 with 5 M NaOH for 1 hr. The pH was then adjusted to 9 with 1 M HCl and the sample was centrifuged. The supernatant was diluted with water to a protein concentration of about 4%. The protein was then hydrolyzed at 50°C by the enzyme alcalase. Vallejo-Cordoba et al. (1986) utilized soy protein hydrolysates for reducing water activity in meat products. Soybean flour was hydrolyzed by acid, enzyme and a combination of acid and enzyme treatment. In the acid hydrolysis, soybean flour (10%) was suspended in 1N H<sub>2</sub>SO<sub>4</sub> and refluxed for 7 hr. In the enzymatic hydrolysis, soybean flour (15%) suspended in water and the pH was then adjusted to 8.3 with 4N KOH. The suspension was incubated for 15 hr with 2.5% (enzyme/substrate) of Alcalase at 50°C or bromelain at 60°C. During the hydrolysis, the pH was maintained at pH 8.3 with

a pH-stat. After hydrolysis, the enzymes were inactivated by heating the suspension at 85°C for 15 min.

## **2. Determination of the Degree of Hydrolysis (DH)**

DH is defined as the percentage of peptide bonds cleaved during hydrolysis (Adler-Nissen, 1976). The number of peptide bonds cleaved during a hydrolysis process can be monitored by determining hydrolysis equivalents which is expressed as equivalents per kg protein (Adler-Nissen, 1986). The hydrolysis equivalents can be measured by determining the increase in free amino groups.

The most widely accepted method to determine the hydrolysis equivalents involves the use of trinitrobenzenesulfonic acid (TNBS) method which determines the concentration of primary amino groups (Adler-Nissen, 1979). The method is a spectrophotometric assay of the chromophore formed by the reaction of TNBS with free amino groups (Figure 8). The reaction takes place under slightly alkaline conditions and is terminated by lowering the pH.

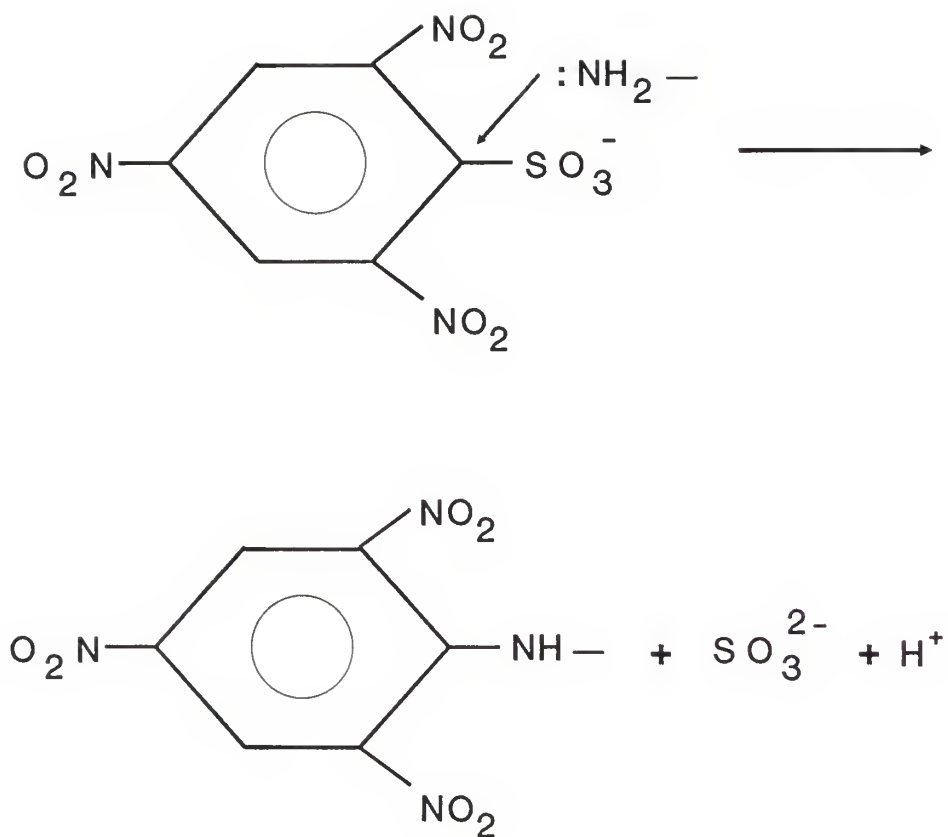


Figure 8. Reaction of TNBS with amino groups  
(Adler-Nissen, 1979)

## MATERIALS AND METHODS

### Materials

All chemicals employed were analytical grade. Wheat gluten (75% protein) and soy protein grade II (91.5% protein) were purchased from United States Biochemical Corporation (Cleveland, OH). Cellulose dialysis tubing (molecular weight cutoff 10,000) was purchased from Spectrum Medical Industries, Inc. (Los Angeles, CA). Egg white (90% protein), L-leucine, papain type II, D-(+)-glucose, 2-thiobarbituric acid, trichloroacetic acid, sodium dodecyl sulfate, 2,4,6-trinitrobenzenesulfonic acid, metmyoglobin type I from horse skeletal muscle, bovine serum albumin, hydrogen peroxide (30% for synthesis) and all other chemicals and materials used were obtained from Sigma Chemical Company (St. Louis, MO).

### Methods

Enzymatic Hydrolysis of Proteins. The methods used to prepare the various MRP for this study are summarized in Figure 9. Egg white, soy protein, or wheat gluten (10%) was suspended in distilled water and the pH was adjusted to 7.0 by the addition of 2.0 N sodium hydroxide (Vallejo-Cordoba et al., 1986) by using a pH meter (Model 671, Markson Science, Inc., Phoenix, AZ). The reaction vessel was a 1000 mL Erlenmeyer flask. The suspensions were incubated for 15 hr with 3% papain (enzyme/substrate) in a water bath (Model 406015, American Optical, Scientific Instrument division, Buffalo, New York) held at  $60 \pm 2$  °C. The 3% papain was suspended in distilled water before adding to the protein suspension. During proteolysis, the pH was maintained at 7.0 manually by

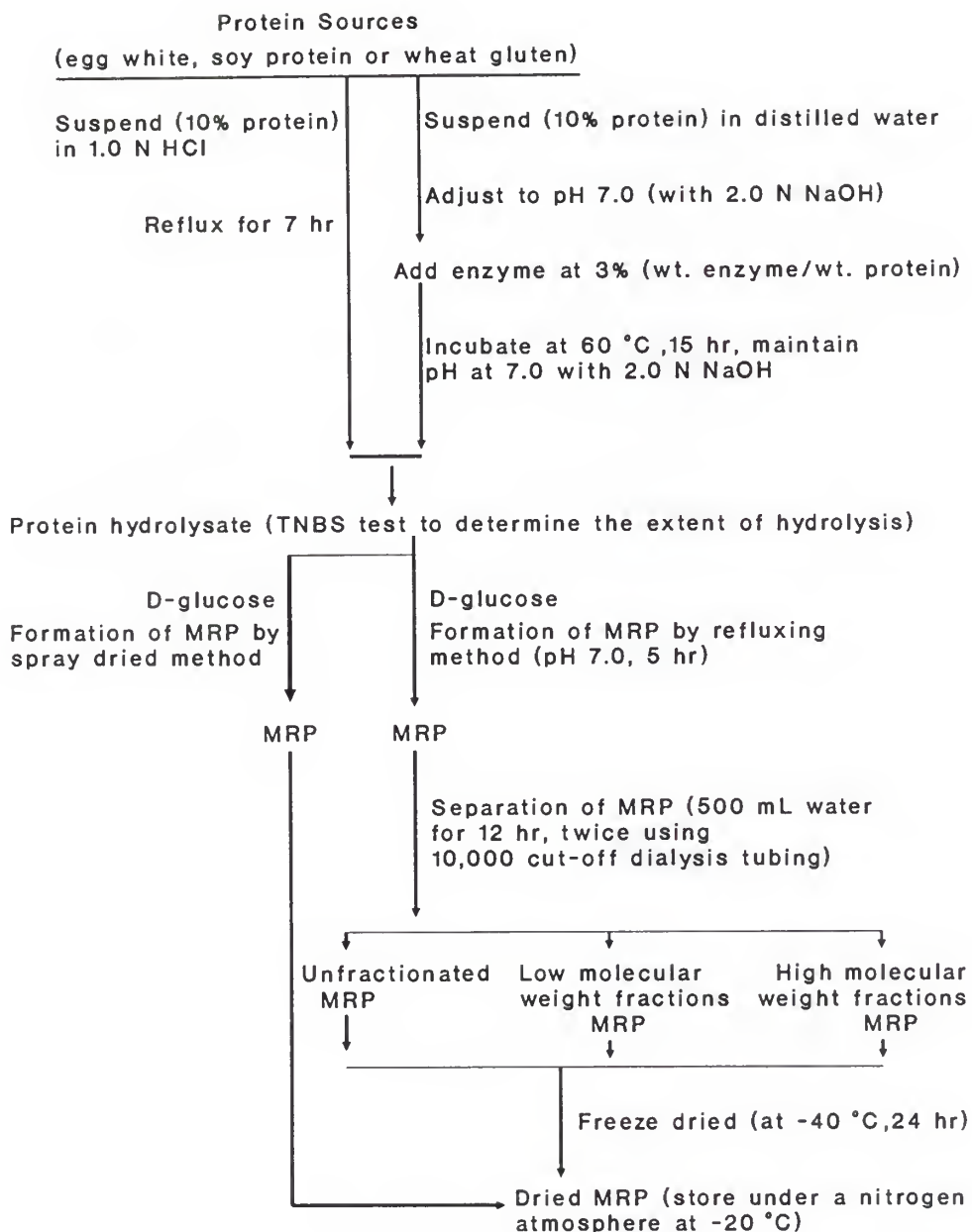


Figure 9. Preparation of Maillard reaction products (MRP) from enzymatic or acid protein hydrolysate with D-glucose by refluxing or spray dried method.

adding 2.0 N sodium hydroxide under constant stirring. The water bath and reaction vessel were covered with aluminum foil to control the temperature and eliminate the loss of vapor. After hydrolysis, the enzyme was inactivated by heating the suspension at 85°C for 15 min. The hydrolysate was stored in an Erlenmeyer flask, flushed with nitrogen and frozen at -20°C.

**Acid Hydrolysis.** Dried egg white (10%) was suspended in 1.0 N hydrochloric acid (HCl) and refluxed for 7 hr (Vallejo-Cordoba et al., 1986) as shown in (Figure 9). The hydrolysate was neutralized with 4.0 N sodium hydroxide. The protein hydrolysate was stored at -20°C under a nitrogen atmosphere in an Erlenmeyer flask.

### **Measurement of the Extent of Hydrolysis**

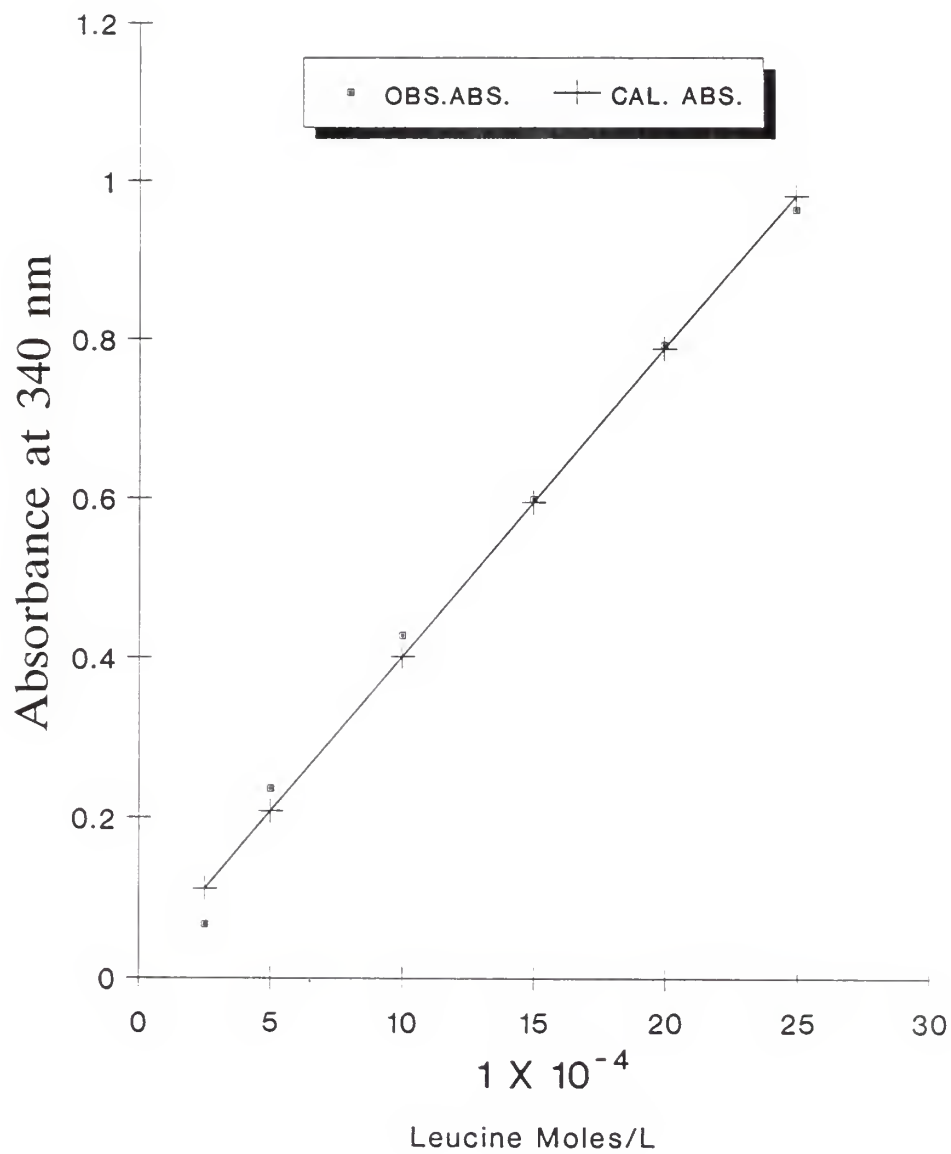
**Preparation of the Standard Curve.** The extent of hydrolysis of the protein was monitored using the TNBS method of Adler-Nissen (1979). The L-leucine standard curve was established to determine the relationship between the concentration of amino equivalents and optical density at 340 nm. A  $2.5 \times 10^{-3}$  M of L-leucine was prepared using 32.8 mg in 100 mL of 1% sodium dodecyl sulfate (NaDodSO<sub>4</sub>). L-leucine solutions of 2.0, 1.5, 1.0, 0.5 and  $0.25 \times 10^{-3}$  M concentrations were prepared from the latter mixture with 1% of NaDodSO<sub>4</sub>. For each concentration, 0.250 mL was added to 2.0 mL of 0.2125 M phosphate buffer (pH 8) in a test tube. The phosphate buffer was prepared by mixing 50 mL of 0.2125 M of sodium phosphate (NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O) solution with 800 mL of 0.2125 M of sodium phosphate dibasic (Na<sub>2</sub>HPO<sub>4</sub>) solution. Two

mL of 0.1% trinitrobenzene sulfonic acid (TNBS) were added to the test tubes. The test tubes were then shaken and covered with aluminum foil to prevent the acceleration of the reaction by exposure to light, and placed in 50°C water bath for 60 min. At the end of 60 min, the reaction was stopped by adding 4 mL of 0.1 N HCl to each test tube. The test tubes were then allowed to stand at room temperature for 30 min before the absorbance reading was taken at 340 nm by using a Perkin-Elmer Spectrophotometer (Model 552, Coleman Instruments Division, Oak Brook, Illinois). A blank was prepared by replacing the L-leucine solution with 1% NaDodSO<sub>4</sub>.

Monitoring the Extent of Hydrolysis. TNBS method was utilized to determine the concentration of primary amine groups in the enzymatic or acidic protein hydrolysate (Adler-Nissen, 1979) using L-leucine standard curve (Figure 10). One mL of protein hydrolysate was suspended in 100 mL of 1% NaDodSO<sub>4</sub> to create a concentration of  $2.5-0.25 \times 10^{-3}$  amino equivalents/L. The diluted protein hydrolysate (0.25 mL) was mixed in a test tube with 2.0 mL of 0.2125 M of phosphate buffer at pH 8. A 0.1% TNBS solution was prepared immediately before use and covered with aluminum foil. Two mL were added to the test tubes and shaken before placing in a water bath at 50°C for 60 min. During incubation, the test tubes were covered with aluminum foil to eliminate the influence of light on the reaction. The reaction was stopped by adding 4.0 mL of 0.1 N HCl and the test tubes were left at room temperature for 30 min. The absorbance of the chromophore was determined using a blank at 340 nm. The blank was prepared by replacing the sample with 1% NaDodSO<sub>4</sub>. The results were expressed as



Figure 10. Relationship between concentration of L-leucine and absorbance at 340 nm (L-leucine standard curve).



amino equivalent per g protein.

### Synthesis of MRP

Spray Dried Method. The enzymatic hydrolysate of egg white, soy protein and wheat gluten containing 0.89, 1.05 and 0.53 amino equiv/g protein, respectively, as well as acid hydrolysate of egg white, soy protein and wheat gluten containing 2.37, 2.90 and 1.47 amino equiv/g protein, respectively, were spray dried by Henningsen Foods, Inc. (Omaha, NE 68144). The hydrolysate protein samples were adjusted to pH 9 with 2.0 N NaOH after the hydrolysis treatment and kept in refrigerator until they were spray dried. They were mixed cold with an equal weight of D-glucose prior to sending to the spray dryer. The spray dryer was indirectly air heated. The inlet, exhaust and outside temperature were 107-121 °C, 67-73 °C and 26 °C, respectively. The air pressure was 17-20 psi and nozzle size was the medium. The dried Maillard reaction products were kept in a bag and stored in a nitrogen atmosphere at -20°C to protect their antioxidative activities.

Refluxing Method. MRP were synthesized by refluxing 25 mL of hydrolyzed egg white, soy protein, wheat gluten, or acid hydrolysate egg white containing 0.89, 1.05, 0.53, and 2.37 amino equiv/g protein, respectively, with an equal weight of D-glucose at 100°C for 5 hr (Lingnert and Lundgren, 1980). The pH of the reaction mixture was adjusted to 7.00 with 0.2125 M of phosphate buffer before starting the reaction. After 5 hr, the MRP were concentrated to viscous liquid using a rotary evaporator (Model 1144131, Brinkmann Instruments,

Inc., Switzerland) at 85°C. The samples to be dried were then placed into round bottom flasks and frozen completely. The MRP were freeze dried by using a lyophilizer (Model 75050, Labconco Corporation, K.C., Mo.) at -40°C for 24 hr. The dried brown pigments were placed into small Erlenmeyer flasks and stored in a nitrogen atmosphere at -20°C to protect their antioxidative activities.

Separation of MRP into Molecular Weight Fractions. In order to obtain molecular weight fractions of the MRP, the pigment preparations were dialyzed against 500 mL deionized-distilled water at room temperature. The dialysis was performed using Spectrapor 6 dialysis tubing with a molecular weight cutoff of 10,000. The water was changed twice, and all the diffusates were retained and combined. The diffusate and dialysate fractions were both concentrated using a rotary evaporator to a viscous liquid at 85°C. The fractions were then freeze-dried at -40°C for 24 hr. The dried pigment fractions were placed in small Erlenmeyer flasks and flashed with nitrogen prior to placing in frozen storage at -20°C.

Isolation of Microsomal Fractions. The microsomal lipid fractions were isolated by the modification procedures of Buege and Aust (1978) and Kanner and Harel (1985) (Figure 11). Samples of meat were removed from beef semimembranosus muscle within 4 hours of slaughter at Kansas State University Meat Laboratory. This muscle was selected because of its likely use in a restructured product. Meat was trimmed of all visible external fat and connective tissue and stored in a nitrogen atmosphere at -20°C for no longer than one week.

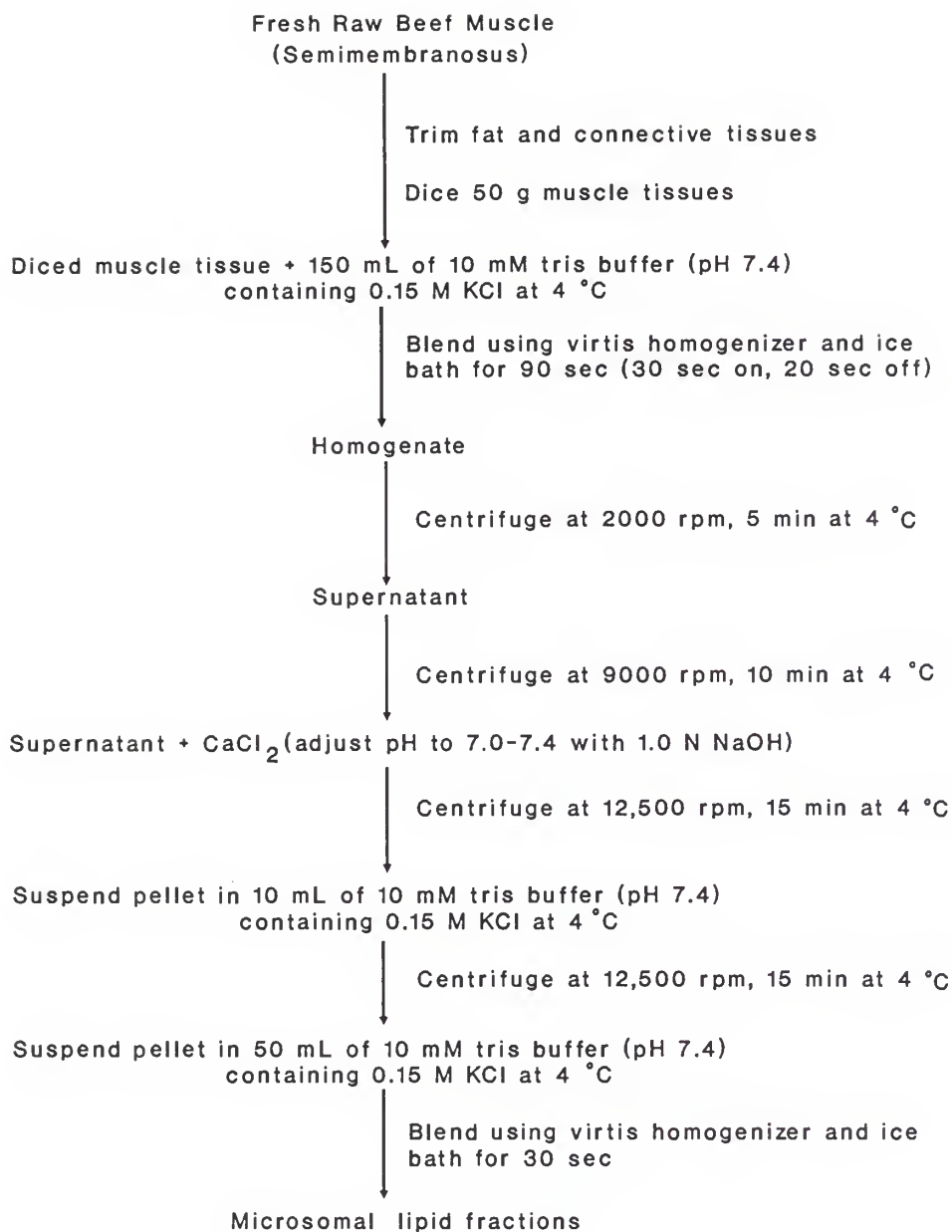


Figure 11. Isolation of microsomal lipid fraction from beef muscle tissue.  
(Buege and Aust, 1978; Harel and Kanner, 1985)

Fifty grams of diced muscle tissue was used to obtain approximately 1 mg microsomal protein/mL in the final solution. The fifty grams diced muscle was blended with 150 mL of 10 mM tris buffer containing 0.15 M KCl (pH 7.40) at 4 °C using an ice bath and a Virtis tissue homogenizer ( Model 6301 0001, the Virtis Company, Gardiner, New York) for 90 sec (30 sec on, 20 sec off). A 10 mM tris buffer was prepared by mixing 10 mM tris acid in 0.15 M KCl with 10 mM tris base in 0.15 KCl until a pH of 7.4 was obtained. The homogenate was then centrifuged (Model J-21C, Beckman Instruments, Inc., Palo Alto, California) at 4°C at 2000 rpm for 5 min. The supernatant obtained was recentrifuged at 9000 rpm for 10 min. Calcium chloride was added to the supernatant to make the concentration 8 mM. The pH was adjusted to 7.0 - 7.4 with 1.0 N NaOH resulting in the precipitation of the microsomal fraction when centrifuged at 12,500 rpm for 15 min. The pellet was then resuspended in 10 mL at 4°C of 10 mM tris buffer (pH 7.4) containing 0.15 KCl and recentrifuged at 12,500 rpm for 15 min. The pellet was then blended with 50 mL of 10 mM tris buffer containing 0.15 M KCl at pH 7.4 using an ice bath with the virtis homogenizer for 30 sec. The microsomal lipid fractions were used immediately after preparation.

**Preparation of the Cooked Meat Model System.** The preparation of the model system is outlined schematically in Figure 12. Ten mL of the microsomal fraction were diluted to 30 mL at 4°C with deionized distilled water. The MRP were incorporated into the model system at a concentration that represented 2 mg/g of intact muscle tissue. The oxidation of the model

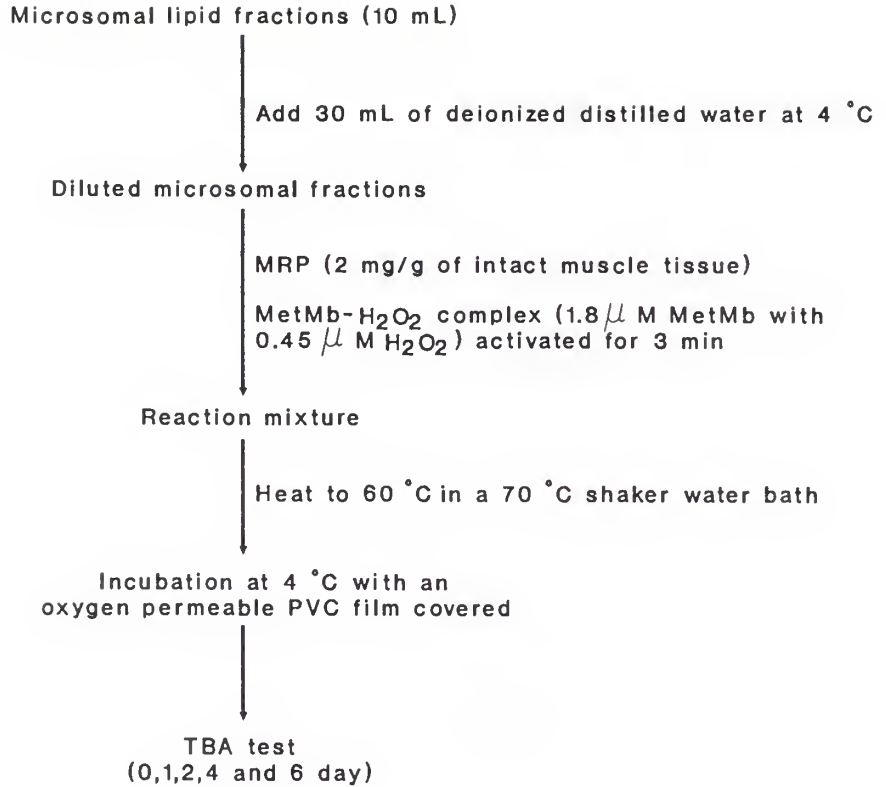


Figure 12. Schematic outline of the experiment for the evaluation of the effect of MRP on the development of lipid oxidation in a cooked meat model system.



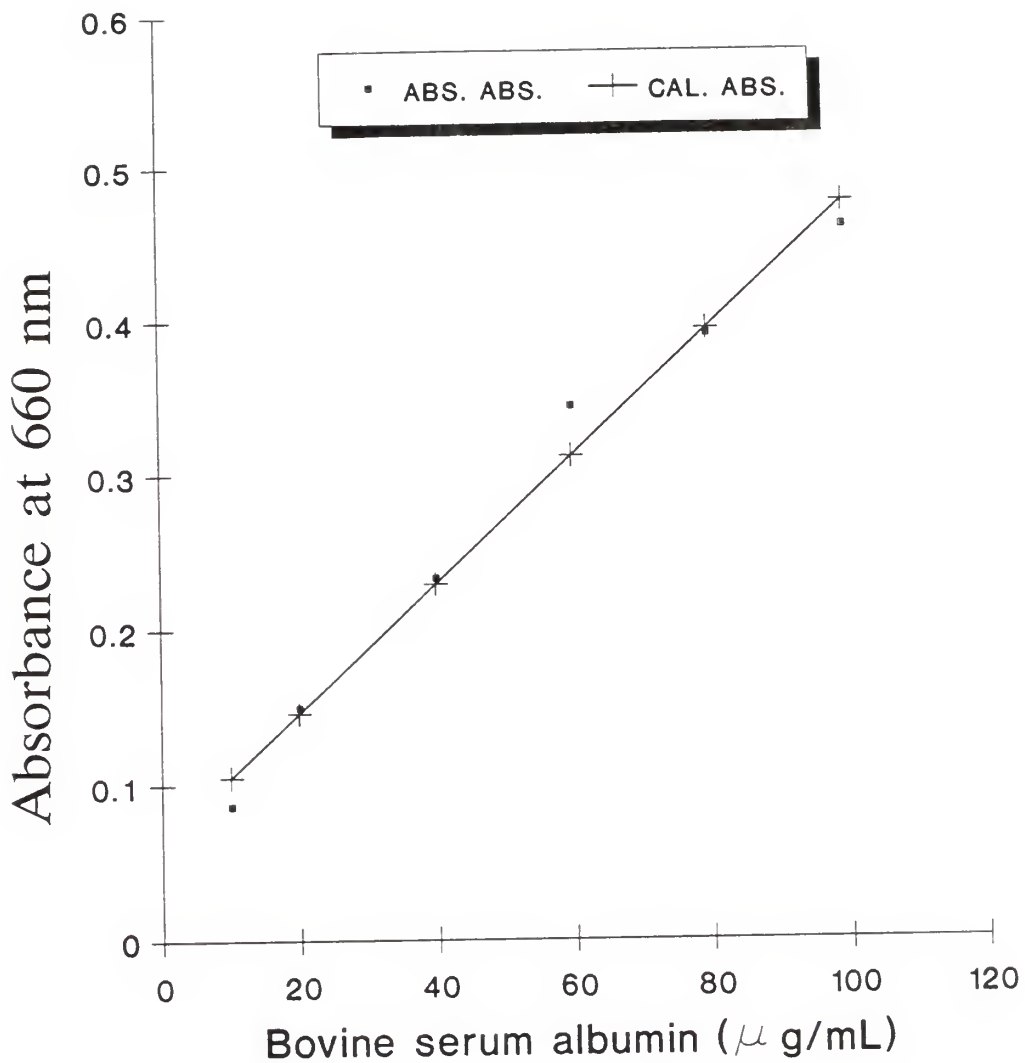
system was initiated by the incubation of metmyoglobin (MetMb) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) at molar ratio of 4:1 ( $1.8 \mu\text{M}$  MetMb with  $0.45 \mu\text{M}$   $\text{H}_2\text{O}_2$ ) for 3 min in 1 mL deionized distilled water. MetMb was added at a concentration of 3mg/g of raw muscle used to obtain the microsomal fraction. The MetMb was dissolved in 1 mL deionized distilled water and mixed with 1 mL of  $0.45 \mu\text{M}$   $\text{H}_2\text{O}_2$  in 50 mL Erlenmeyer flask. After 3 min, the diluted microsomal fraction was added to the MetMb- $\text{H}_2\text{O}_2$  reactive compound. The combined solutions were heated to  $60^\circ\text{C}$  in a  $70^\circ\text{C}$  shaker water bath. After heating, the samples were stored in the refrigerator covered with oxygen permeable PVC film. An initial TBA value was determined immediately after removing the sample from the water bath with subsequent readings being taken at 1, 2, 4, and 6 days. Protein content of microsomal fraction was determined immediately after the sample was removed from the water bath. This method is described below. The blank was composed of 10 mL of deionized distilled water, 10 mL of 10 mM tris buffer in 0.15 M KCL (pH 7.4), 2mg/g MRP, 3mg/g MetMb and 1 mL of diluted  $\text{H}_2\text{O}_2$ . The blank and samples were treated in an identical manner.

**Thiobarbituric Acid Value Determination.** The extent of lipid oxidation in the incubated samples of the model lipid system was measured by the thiobarbituric acid (TBA) test as described by Buege and Aust (1978). One mL of the model system was mixed thoroughly with 2 mL of TBA reagent. The TBA reagent was prepared by dissolving 15% trichloroacetic acid, 0.375% thiobarbituric acid and 0.25 N hydrochloric acid. The mixture was heated for 15 min in a boiling water bath and centrifuged at 1000 rpm for 10 min. The absorbance of the

sample was determined at 535 nm against a blank containing all reagents except the microsomal fraction. The results were expressed as nmol malonaldehyde per mg protein using a molar extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

**Microsomal Protein Determination.** The modified Lowry procedure (Markwell et al., 1978) was used to determine the protein content of the microsomal fraction by using bovine serum albumin as standard (Figure 13). Two reagents labelled A and B were prepared. Reagent A was composed of 2.0%  $\text{Na}_2\text{CO}_3$ , 0.4% NaOH, 0.16% sodium tartrate and 1% sodium dodecyl sulfate. Reagent B was prepared by making a 4% cuprous sulfate solution ( $\text{CuSO}_4 \cdot 5 \text{ H}_2\text{O}$ ). Reagent C was then prepared by mixing 100 parts of reagent A with 1 part of reagent B. One mL of microsomal fraction was added to 3 mL of reagent C and incubated at room temperature for 15 min. The samples were mixed thoroughly with 0.3 mL of Folin-Ciocalteu's phenol reagent which had been diluted 1:1 with distilled water. The samples were incubated for 45 min at room temperature before the absorbance readings were taken at 660 nm against a blank containing all reagents minus the microsomal fraction. The results were expressed as mg microsomal protein per mL microsome fraction.

Figure 13. Relationship between concentration of bovine serum albumin and absorbance at 660 nm (modified Lowry procedure standard curve).



## RESULTS AND DISCUSSION

### Antioxidative Activity of Unfractionated MRP from Various Protein Sources

Egg white, soy protein and wheat gluten were selected as the protein sources in this investigation since they are economical and readily available. Enzymatic treatment of the three protein sources was performed to determine which would produce MRP possessing the greatest antioxidant activity in a cooked beef model system. Papain was selected as the protease because of its less specific and rapid endopeptidase activity (Hill and Schmidt, 1962) as well as because of its ability to produce a higher proportion of small soluble peptides than other proteases (Clegg and Manson, 1974).

The extent of hydrolysis of the food proteins used in this study was monitored by reacting the free amine groups of the peptides with TNBS as described by Alder-Nissen (1979). The results of reacting the enzymatic hydrolysate as well as the hydrolysate resulting from acid treatment of the proteins is shown in Table 1. Increasing values indicated a more complete hydrolysis of the food proteins. Hydrolysis of the proteins was more extensive using an acid treatment than the enzymatic treatment for each protein source. Differences in the extent of hydrolysis of the various proteins using identical treatments is most likely due to the different amino acid sequences which dictate susceptibility of the peptide

Table 1. The extent of hydrolysis of egg white, soy protein and wheat gluten (TNBS values).

Treatments	Protein sources	TNBS values
Enzymatic hydrolysis	Egg white	0.89
	Soy protein	1.05
	Wheat gluten	0.53
Acid hydrolysis	Egg white	2.37
	soy protein	2.90
	Wheat gluten	1.47

TNBS values = amino equivalents per g protein.

linkages to the protease.

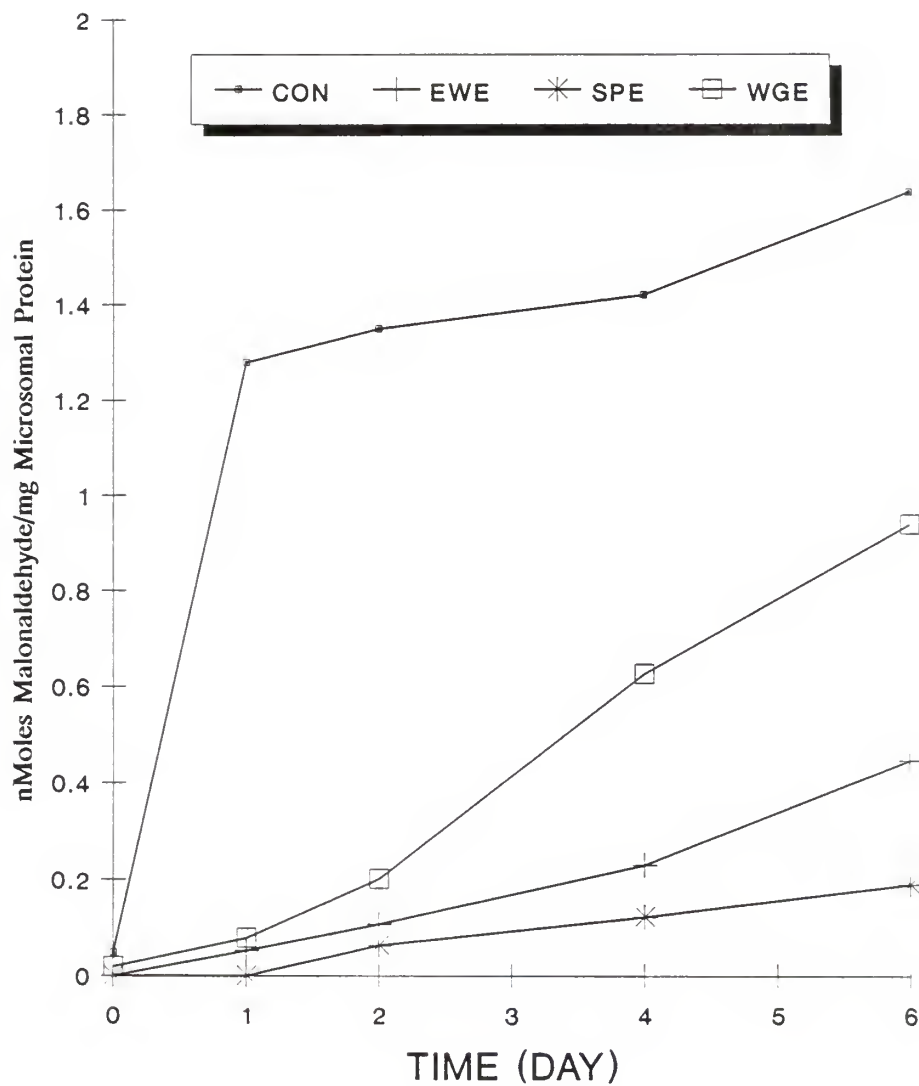
The efficacy of unfractionated MRP derived from the enzymatic hydrolysate of egg white, soy protein and wheat gluten and subsequent refluxing with D-glucose as an antioxidant in a cooked meat model system is shown in Figure 14. The model system consisted of the microsomal fraction of semimembranosus beef tissue and activated metmyoglobin (MetMb). The microsomal fraction of this muscle was utilized in the model system because it is rich in cellular membrane material such as the ribosomes (Lehninger, 1982). These membranes are composed primarily of unsaturated phospholipids and therefore will oxidize readily. In addition, the oxidation of the membrane lipids has been demonstrated to be the primary source of oxidation products associated with WOF (Igene and Pearson, 1979).

Metmyoglobin was activated to a lipid oxidation catalyst by reacting it with  $H_2O_2$  prior to their addition to the model system. The TBA value resulting from the oxidation of the heated microsomal membrane lipids catalyzed by activated MetMb and containing no MRP (CON) at zero time was approximately 0.05 (Figure 14). There was a 24-fold increase in the concentration of TBA reactive lipid oxidation products after 1 day storage followed by a more gradual increase during the subsequent six days of storage. These results support the hypothesis of Harel and Kanner (1985a). These researchers



Figure 14. The antioxidative activity of unfractionated MRP on the development of lipid oxidation (as measured by the TBA test) in cooked microsomal membrane lipids from beef tissues stored at 4°C.

CON = control, EWE = egg white enzymatic hydrolysate, SPE = soy protein enzymatic hydrolysate, WGE = wheat gluten enzymatic hydrolysate.



proposed that MetMb is activated by  $H_2O_2$  in a reaction that results in the formation of a porphyrin cation radical which serves as an initiator of lipid oxidation. These authors observed that the addition of low concentrations of the metal chelator EDTA did enhance the antioxidative activity of ascorbic acid, indicating that the release of iron from meat pigments also plays a role in the catalysis of lipid oxidation in cooked meat.

In the samples containing added unfractionated MRP, the oxidation of the microsomal membrane lipids was influenced by the type of protein hydrolysate utilized to produce the MRP. The unfractionated MRP prepared by refluxing the soy protein enzymatic hydrolysate with D-glucose (SPE) was more effective in retarding lipid oxidation than the MRP formed by refluxing the enzymatic hydrolysate of egg white and D-glucose (EWE). However, the difference in these antioxidative activities was not large. The MRP derived from the enzymatic hydrolysis of wheat gluten (WGE) had the least antioxidant activity among the pigment preparations. The pattern of antioxidant activity displayed by the various MRP was parallel to the extent of protein hydrolysis that occurred in their preparation (Table 1). Thus, it appears that the greater extent of hydrolysis among the enzymatic preparations may result in MRP with greater antioxidant activity.

Previous work in this field has demonstrated that amino acids differ in their ability to form effective antioxidants when refluxed with monosaccharides. Lingnert and Eriksson (1980) investigated the antioxidative effect of MRP produced by refluxing six different amino acids with three different sugars. The most effective antioxidants were synthesized from mixtures of arginine-xylose and lysine-xylose. Histidine formed more effective antioxidative browning compounds in combination with various sugars than did valine, cysteine or glutamic acid. This study, as well as others (Eriksson et al., 1971), demonstrated that amino acids with free amine groups on the side chain form the most potent antioxidants. A possible antioxidative mechanism is that the amine-containing amino acids may be reacting with the carbonyls produced during the breakdown of lipid peroxides. Thus, these carbonyls become unavailable to react with TBA or for direct measurement by gas chromatography. In the present study, the three protein sources differed in their concentrations of amino acids possessing amine-containing side chains (Table 2). The soy protein isolates contained the highest collective concentration of lysine, arginine and histidine, followed by the egg white preparation and the wheat gluten. The order of antioxidative efficiency of the MRP formed from these protein sources was identical in order, with the soy protein isolate being the most efficient and wheat gluten being the least. These results may indicate that the basic amino acid

Table 2. Concentration of basic amino acids in 100 grams of various protein isolates from food.

	Amino Acid	Grams
I. Egg White Protein - Dried	Lysine	5.54
	Arginine	5.26
	Histidine	2.04
	Total Basic	12.84
II. Soy Protein Isolate	Lysine	5.56
	Arginine	6.49
	Histidine	2.25
	Total Basic	14.30
III. Wheat Gluten	Lysine	2.30
	Arginine	3.89
	Histidine	1.74
	Total Basic	7.93

concentration of the protein is directly related to the antioxidative capacity of the Maillard product formed from their hydrolysate.

Lingnert and Eriksson (1980a) obtained protein hydrolysates from enzymatically treated malt sprouts, brewer's grain and bovine erythrocytes. Of the various protein hydrolysates, the malt hydrolysate was observed to form the strongest antioxidants when reacted with glucose. All of the protein hydrolysates were less effective antioxidants alone as compared to their antioxidative effects as MRP. However, the MRP formed from the reaction of protein hydrolysates were found to be inferior in comparison to those formed from the reaction of a single amino acid and a monosaccharide. Interestingly, these researchers found that identical proteins would form MRP of differing antioxidant activity depending upon the enzyme that was utilized during the hydrolysis. Again, the sequence of amino acids or the terminal acid in a peptide appears to influence the potency of the MRP as an antioxidant.

#### **Influence of High Molecular Weight Fraction on Antioxidative Activity of MRP**

MRP synthesized from various protein sources were fractionated by molecular weight using dialysis tubing. The influence of high molecular weight fractions (>10,000) of MRP obtained from the enzymatic hydrolysate of the three protein

sources and subsequent refluxing with D-glucose on TBA values of cooked microsomal lipids is presented in Figure 15. The results showed that the model system containing added high molecular weight fractions of MRP prepared from refluxing wheat gluten (WGEH), egg white (EWEH) or soy protein enzymatic hydrolysate (SPEH) and D-glucose were roughly equally effective with soy protein being perhaps slightly more effective. The high molecular weight fractions of the MRP from each protein source were less effective as antioxidants than the corresponding unfractionated MRP. For example, all three unfractionated MRP held the TBA values below 1.0 after six days (Figure 14), while none of the high molecular weight fractions could hold the TBA values below this mark after six days (Figure 15).

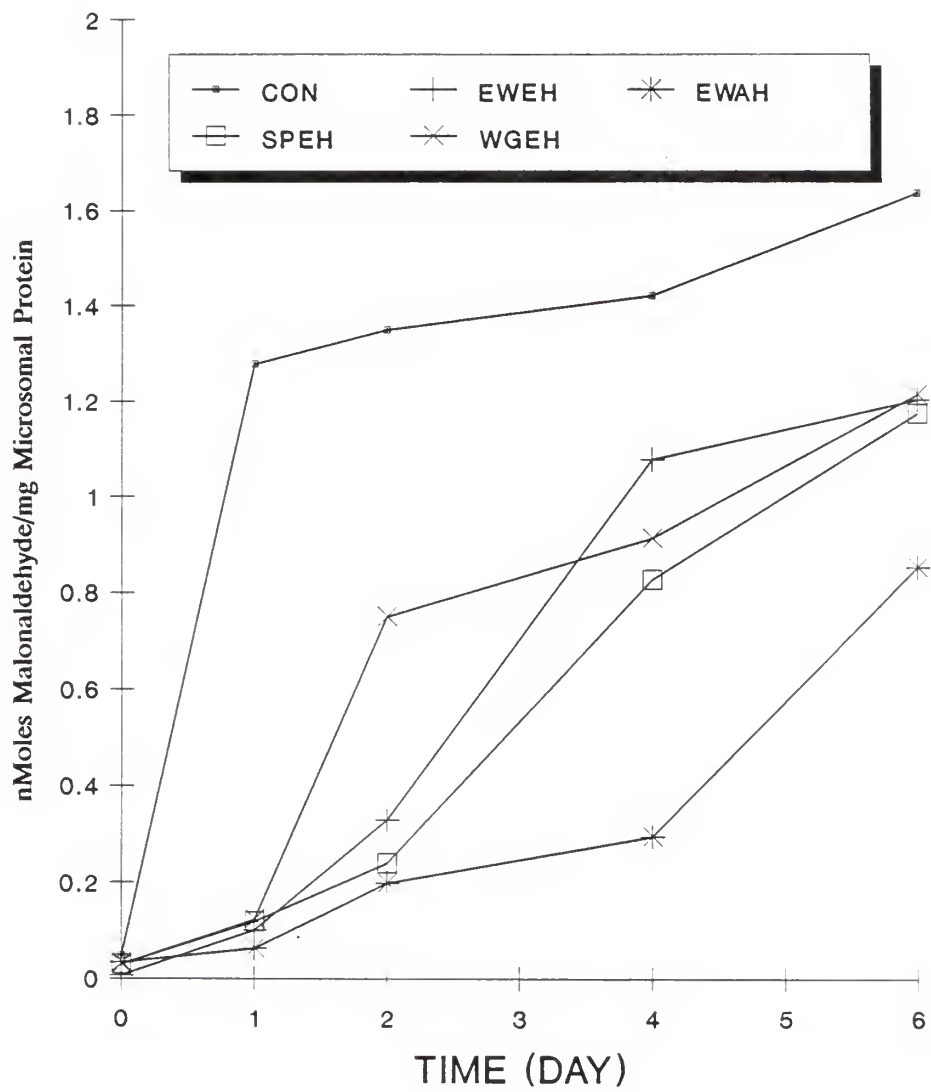
In order to analyze the effect of the extent of hydrolysis on the formation of antioxidative MRP, the various protein sources used in this study were treated with acid rather than enzymes. This treatment resulted in more extensive hydrolysis of the various proteins (Table 1). The high molecular weight fraction of MRP derived from egg white acid hydrolysate (EWAH, Figure 15) was the most effective among those fraction at delaying the onset of lipid oxidation. This may be due to the increased exposure of free primary amine groups available to react with carbonyls produced as a result of lipid oxidation.

These observations may indicate that the predominant



Figure 15. The antioxidative activity of high molecular weight MRP fraction on the development of lipid oxidation (as measured by the TBA test) in cooked microsomal membrane lipids from beef tissues stored at 4°C.

CON = control, EWEH = egg white enzymatic hydrolysate, high molecular weight fraction, EWAH = egg white acid hydrolysate, high molecular weight fraction, SPEH = soy protein enzymatic hydrolysate, high molecular weight fraction, WGEH = wheat gluten enzymatic hydrolysate, high molecular weight fraction.



antioxidants of MRP are the low molecular weight components, and therefore, increased heat treatments would diminish the antioxidative property of MRP.

#### **Effect of Low Molecular Weight Fractions of MRP**

The TBA values for samples treated with low molecular weight MRP fractions (<10,000) synthesized from three protein sources hydrolyzed with papain are presented in Figure 16. A comparison of antioxidant activity among the enzymatic hydrolysate of various proteins showed that the low molecular weight fractions of MRP formed from soy protein (SPEL) was the strongest antioxidant, followed by that of egg white (EWEL) and finally that of wheat gluten (WGEL). In a comparison of the antioxidative activity of low molecular weight MRP fraction with that of unfractionated pigment for each individual protein source, the unfractionated MRP exhibited only slightly more inhibitory effect on the lipid oxidation of the model system than the corresponding low molecular weight fraction (Figure 17). Also, the low molecular weight MRP fraction for each individual protein sources exhibited greater inhibitory effect on the lipid oxidation of the model system than the corresponding high molecular weight fraction (Figure 18). Thus, the antioxidant activities of the MRP appear to be primarily due to the low molecular weight constituents of the MRP.

The results of this study agree with other researchers

Figure 16. The antioxidative activity of low molecular weight MRP fraction on the development of lipid oxidation (as measured by the TBA test) in cooked microsomal membrane lipids from beef tissues stored at 4°C.

CON = control, EWEL = egg white enzymatic hydrolysate, low molecular weight fraction, EWAL = egg white acid hydrolysate, low molecular weight fraction, SPEL = soy protein enzymatic hydrolysate, low molecular weight fraction, WGEL = wheat gluten enzymatic hydrolysate, low molecular weight fraction.

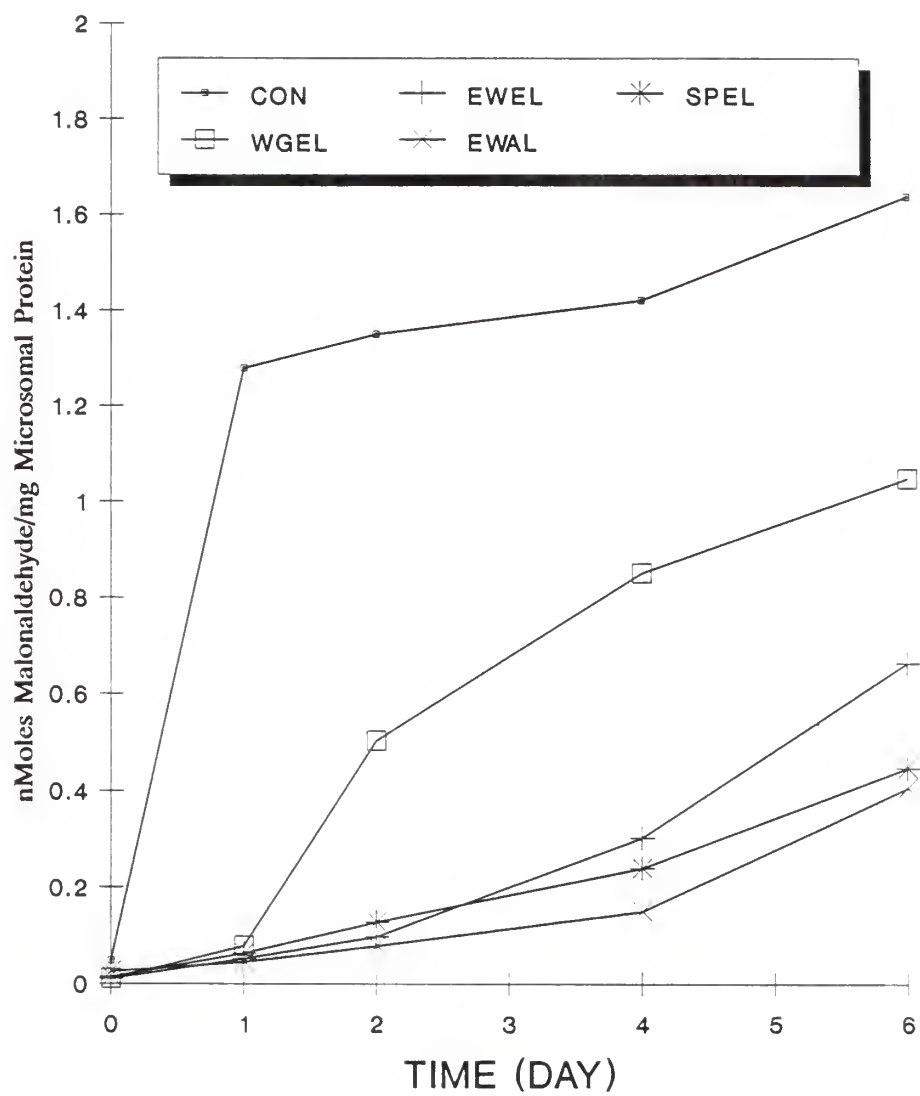


Figure 17. A comparison of the antioxidative activity of low molecular weight MRP fractions with unfractionated MRP on the development of lipid oxidation (as measured by the TBA test) in cooked microsomal membrane lipids from beef tissues stored at 4°C.

CON = control, EWE = egg white enzymatic hydrolysate, EWEL = egg white enzymatic hydrolysate, low molecular weight fraction, SPE = soy protein enzymatic hydrolysate, SPEL = soy protein enzymatic hydrolysate, low molecular weight fraction, WGE = wheat gluten enzymatic hydrolysate, WGEL = wheat gluten enzymatic hydrolysate, low molecular weight fraction.

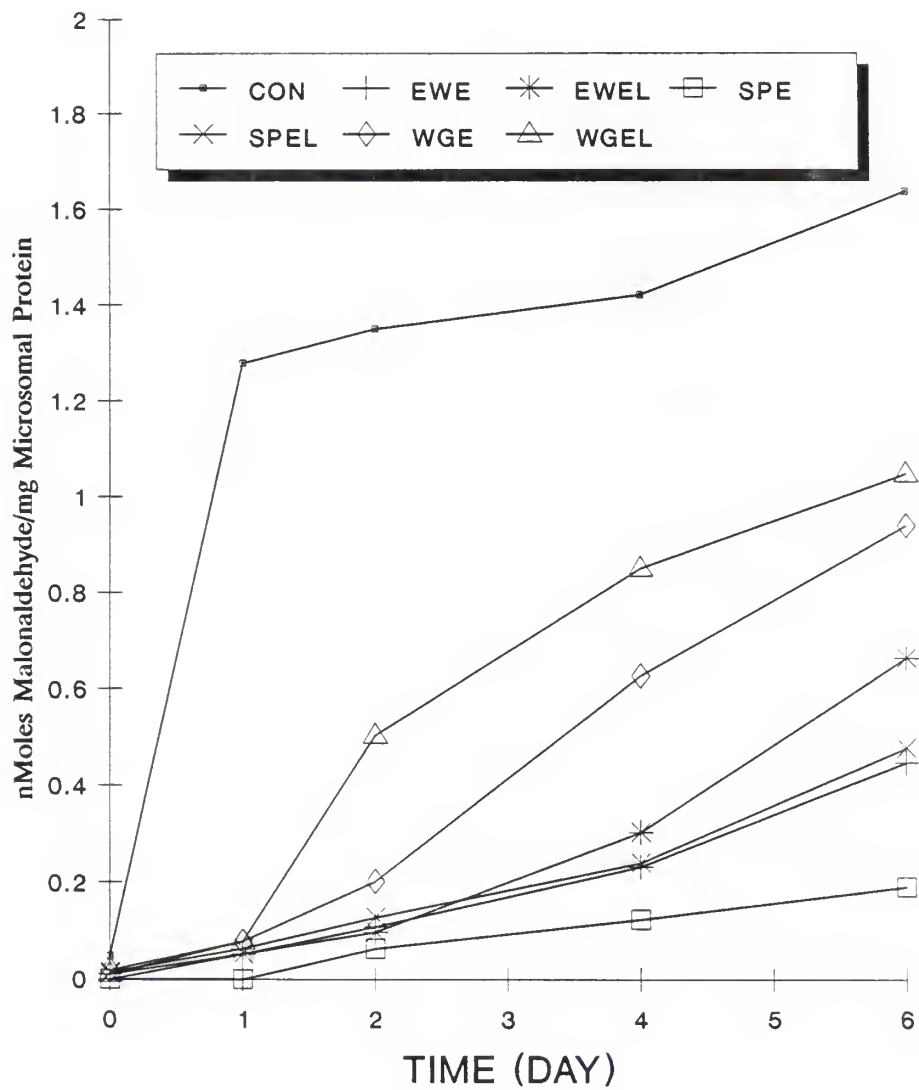
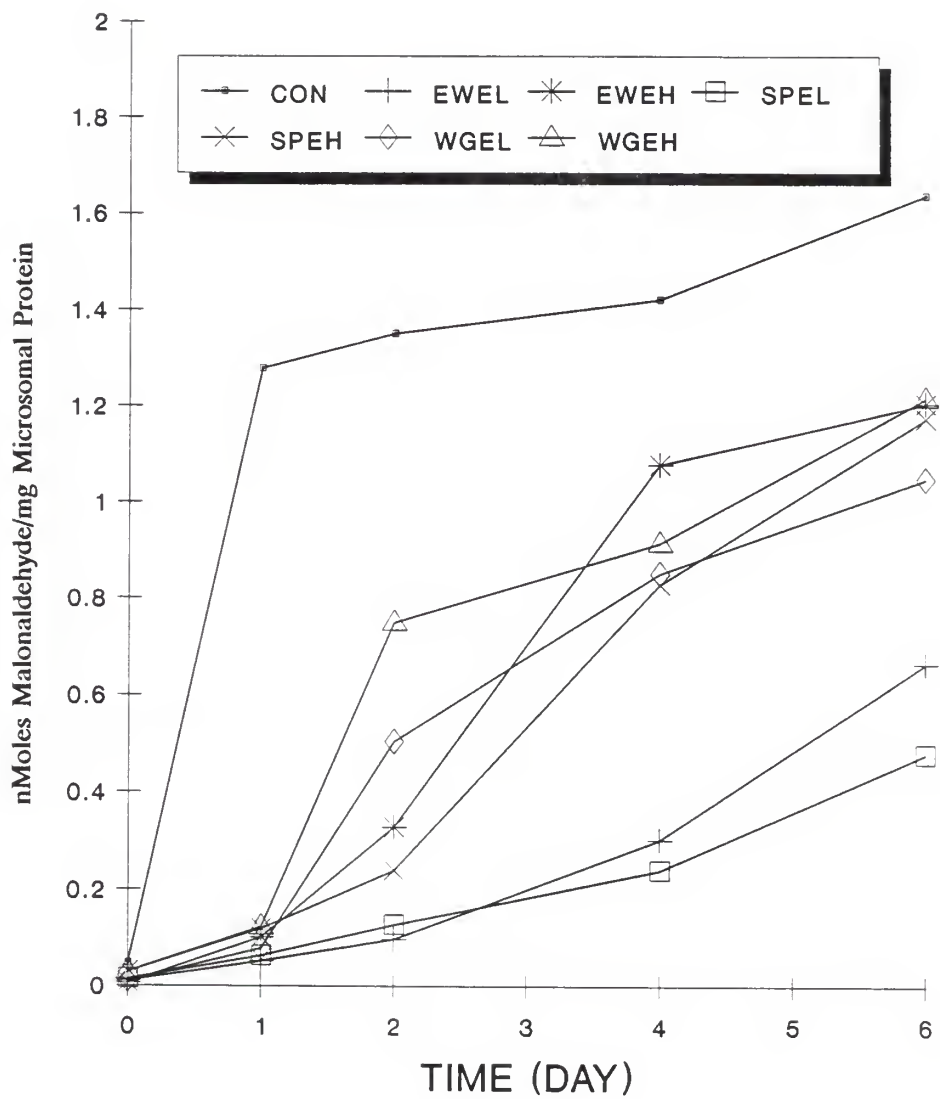


Figure 18. A comparison of the antioxidative activity of low molecular weight MRP fractions with high molecular weight MRP fractions on the development of lipid oxidation (as measured by the TBA test) in cooked microsomal membrane lipids from beef tissues stored at 4°C.

CON = control, EWEL = egg white enzymatic hydrolysate, low molecular weight fraction, EWEH = egg white enzymatic hydrolysate, high molecular weight fraction, EWAL = egg white acid hydrolysate, low molecular weight fraction, EWAH = egg white acid hydrolysate, high molecular weight fraction, SPEL = soy protein enzymatic hydrolysate, low molecular weight fraction, SPEH = soy protein enzymatic hydrolysate, high molecular weight fraction, WGEL = wheat gluten enzymatic hydrolysate, low molecular weight fraction, WGEH = wheat gluten enzymatic hydrolysate, high molecular weight fraction.





who have worked in this area. For example, Sato et al. (1973) extracted the MRP from retorted (115.5 °C) beef and isolated the low molecular fraction by dialysis. This fraction was more effective in preventing WOF in cooked ground beef stored at 4 °C for 48 hours. Yamaguchi et al. (1981) obtained MRP molecular weight fractions by refluxing D-xylose and glycine for two hours and separating them by size exclusion chromatography. One fraction having a molecular weight of 4500 daltons was particularly effective at inhibiting the oxidation of a linoleic acid model system.

The antioxidative mechanism and the compounds responsible for this effect have not yet been clearly elucidated. Evans et al. (1958) were the first to demonstrate that reductones produced as a result of the Maillard reaction could inhibit the development of lipid oxidation. Examples of these compounds are triose-reductone, dihydroxymaleic acid and reductic acid. These compounds share a common structural feature in that they are enediols and thus, like common commercial antioxidants, can act as reducing agents while forming stable free radicals due to resonance. Eichner (1975) also concluded that the colorless intermediates of MRP are essential to the antioxidative effect of these products. Compounds such as 1,2-enaminoles produced from the Amadori mechanism were found to efficiently quench free radicals and inhibit lipid oxidation.

It is evident from Figure 17 that the unfractionated MRP

produced in this study do contribute to the overall antioxidative effect of these pigments. This antioxidative effect of the high molecular weight fraction may be due to reductone-like structures within the complex ringed structure of the brown pigments, or may be due to a metal complexing capability of the large molecular weight fractions as postulated by some authors (Bailey et al., 1987).

The low molecular weight fraction of MRP derived from the acid hydrolysate of egg white again exhibited greater inhibition of the development of lipid oxidation on the model system than that of prepared from the egg white enzymatic hydrolysate (Figure 16). While the concentrations of reductone-like compounds were not analyzed in this study, it is possible that the greater extent of hydrolysis of the various proteins removes stearic hindrances that favor the formation of higher concentrations of reductones.

#### MRP of Egg White Enzymatic Hydrolysate vs MRP of Egg White Acid Hydrolysate

In order to investigate the effect of more extensive hydrolytic conditions on the formation of antioxidative MRP, an acid hydrolysis of the proteins was carried out. Hydrochloric acid (1.0 N HCl) was utilized as the hydrolysis medium because it has been reported to be commercially preferable (Pendergast, 1974).

The efficacy of the MRP formed from either acid (EWA) or

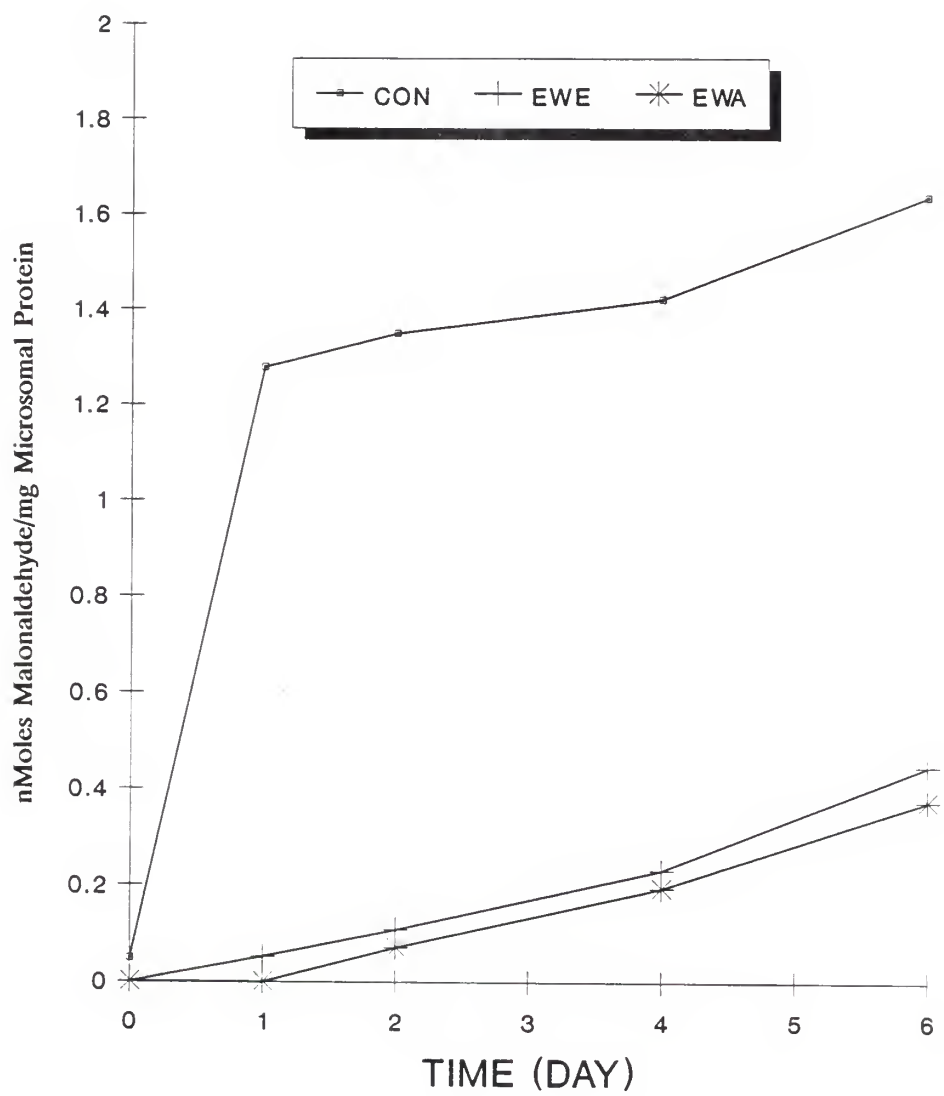
enzymatic hydrolysate of egg white (EWE) and subsequent refluxing with D-glucose in retarding lipid oxidation in the model system is shown in Figure 19. The results demonstrate that the MRP of egg white acid hydrolysate was slightly more effective than that of egg white enzymatic hydrolysate. More dramatic differences were observed between the acid and enzymatically prepared high molecular weight fractions (Figure 15) and low molecular weight fractions (Figure 16) of egg white with the acid preparation being more effective each time. A possible explanation for this apparent discrepancy of these results may lie in the antioxidative mechanisms that work for each molecular weight fraction. Much like the antioxidative synergism exerted by the metal chelator citric acid and the free radical quenching BHA, the low and high molecular weight fractions of the MRP produced from these hydrolysates may enhance each others effectiveness. The net effect of this synergism is that the unfractionated MRPs produced from enzymatic or acid hydrolyzed egg white protein exert a similar total antioxidative effect even though the individual fractions produced very different levels of oxidation inhibition.

#### MRP of Egg White Acid Hydrolysate vs Egg White Acid Hydrolysate

The influence of acid hydrolysate of egg white without forming the MRP (EWW) and MRP derived from the acid hydrolysis

Figure 19. A comparison of the antioxidative activity of MRP prepared from enzymatic hydrolysate with those prepared from acid hydrolysate on development of lipid oxidation (as measured by the TBA test) in cooked microsomal membrane lipids from beef tissues stored at 4°C.

CON = control, EWE = egg white enzymatic hydrolysate, EWA = egg white acid hydrolysate.



of egg white and subsequent refluxing with D-glucose (EWA) on the development of lipid oxidation in the model system is presented in Figure 20. These results indicate that some antioxidative effect was achieved by the use of acid hydrolysis of egg white without forming the MRP. This effect was most likely due to the reaction of carbonyls produced from lipid oxidation with the free amines of the protein hydrolysate. This reaction would be identical to that in the early stages of Maillard browning. However, a much greater antioxidative effect was observed using the MRP obtained from refluxing the protein hydrolysates and glucose. These results are similar to those of Bishov et al. (1977), who reported that protein hydrolysates alone were effective antioxidants in a freeze-dried model system. In addition, the results from the present study confirms the work of Lingnert and Eriksson (1980a), who found that protein hydrolysates themselves exhibit some antioxidative effect. However, these researchers also observed that the formation of browning pigments was essential to forming more effective antioxidative compounds.

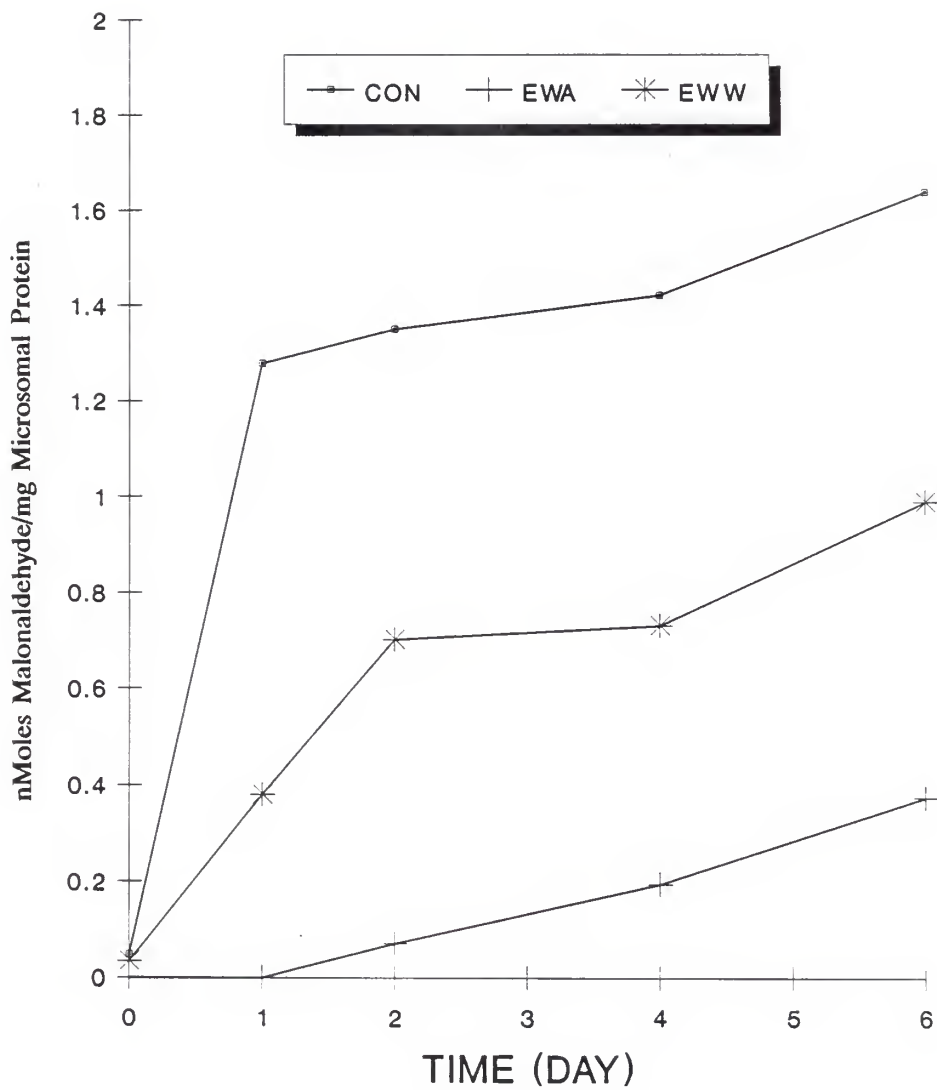
#### The Influence of MRP Synthesized from Acid Protein Hydrolysates and Glucose by Spray Drying

The results of this investigation has pointed to the potential antioxidative effects of the low molecular weight and thus early MRP. Therefore, spray drying of a protein hydrolysate and glucose solution was conducted as a potential

Figure 20. A comparison of the antioxidative activity of MRP prepared from acid hydrolysate with acid protein hydrolysate themselves on development of lipid oxidation (as measured by the TBA test) in cooked microsomal membrane lipids from beef tissues stored at 4°C.

CON = control, EWA = egg white acid hydrolysate, EWW = egg white acid hydrolysate without glucose and refluxing treatment.



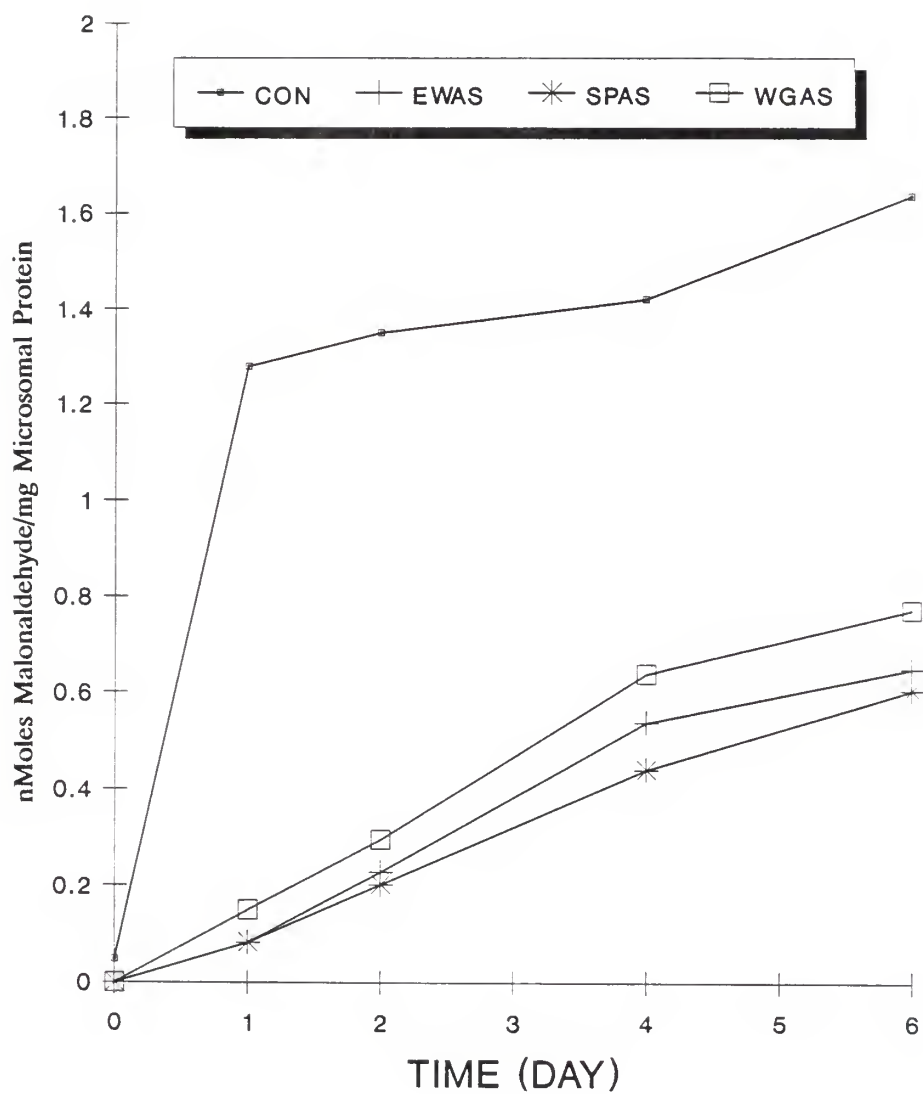


method for producing antioxidative MRP on a commercial scale. Each of the protein sources utilized in this study was hydrolyzed by both acid (1.0 HCl) and enzyme (papain).

The antioxidative property of MRP derived from the acid hydrolysis of three protein sources and subsequent spray-drying with D-glucose on a cooked meat model system is shown in Figure 21. The results demonstrate that the MRP produced from spray dried egg white (EWAS) or soy protein acid hydrolysate (SPAS) were roughly equally effective in delaying the development of lipid oxidation. The MRP prepared from wheat gluten acid hydrolysate (WGAS) was slightly less effective than the other protein sources. In comparing the efficiency of MRP produced by spray drying egg white protein acid hydrolysate with that of egg white hydrolysate alone or the MRP produced by refluxing (Figure 19), the spray dried product appears to lie in between in terms of antioxidative efficiency. Thus, the spray drying heat treatment does produce some early Maillard products which may be serving as free radical quenchers and are effectively delaying lipid oxidation. However, the lack of high molecular weight browning polymers in the spray dried product may result in prooxidative metals that are not chelated and thus are free to become involved in catalysis of lipid oxidation. In this model system, free iron probably is released from MetMb during the heating process.

Figure 21. The antioxidative activity of MRP synthesis from acid protein hydrolysate with glucose by spray dried method on the development of lipid oxidation (as measured by the TBA test) in cooked microsomal membrane lipids from beef tissues stored at 4°C.

CON = control, EWAS = egg white acid hydrolysate, SPAS = soy protein acid hydrolysate, WGAS = wheat gluten acid hydrolysate.



### The Influence of MRP Synthesized From Enzymatic Protein Hydrolysates and Glucose by Spray Drying

The antioxidative effect of the MRP obtained from the enzymatic hydrolysis of the various protein sources and subsequent spray-drying with D-glucose on the cooked meat model system is shown in Figure 22. In a comparison of the antioxidative activity of MRP derived from acid hydrolysate with that from enzymatic hydrolysate, MRP produced from the acid hydrolysates exhibited more inhibitive effect than MRP derived from the enzymatic hydrolysates. Again, the greater degree of hydrolysis affected by the acid may be responsible for the production of more potent antioxidative MRPs due to the exposure of more peptide amine groups.

### The Relative Antioxidative Efficacy of MRP Produced by Spray-drying vs that Produced by Refluxing

A comparison of the antioxidative efficiency of MRP derived from egg white and glucose by the spray-dried method versus that produced by refluxing is shown in Figure 23. Using either type of hydrolysate (enzymatic or acid), the MRP derived as a result of refluxing (EWAR or EWER) was demonstrated to be the more potent antioxidant in the model system. The MRPs produced by spray drying (EWAS or EWES) did not effectively hold the TBA values below 0.5 after four days. Although the spray-dried products certainly contain reductones and other antioxidative Maillard browning intermediates, the

Figure 22. The antioxidative activity of MRP synthesis from enzymatic protein hydrolysate with glucose by spray dried method on the development of lipid oxidation (as measured by the TBA test) in cooked microsomal membrane lipids from beef tissues stored at 4°C.

CON = control, EWES = egg white enzymatic hydrolysate, SPES = soy protein enzymatic hydrolysate, WGES = wheat gluten enzymatic hydrolysate.

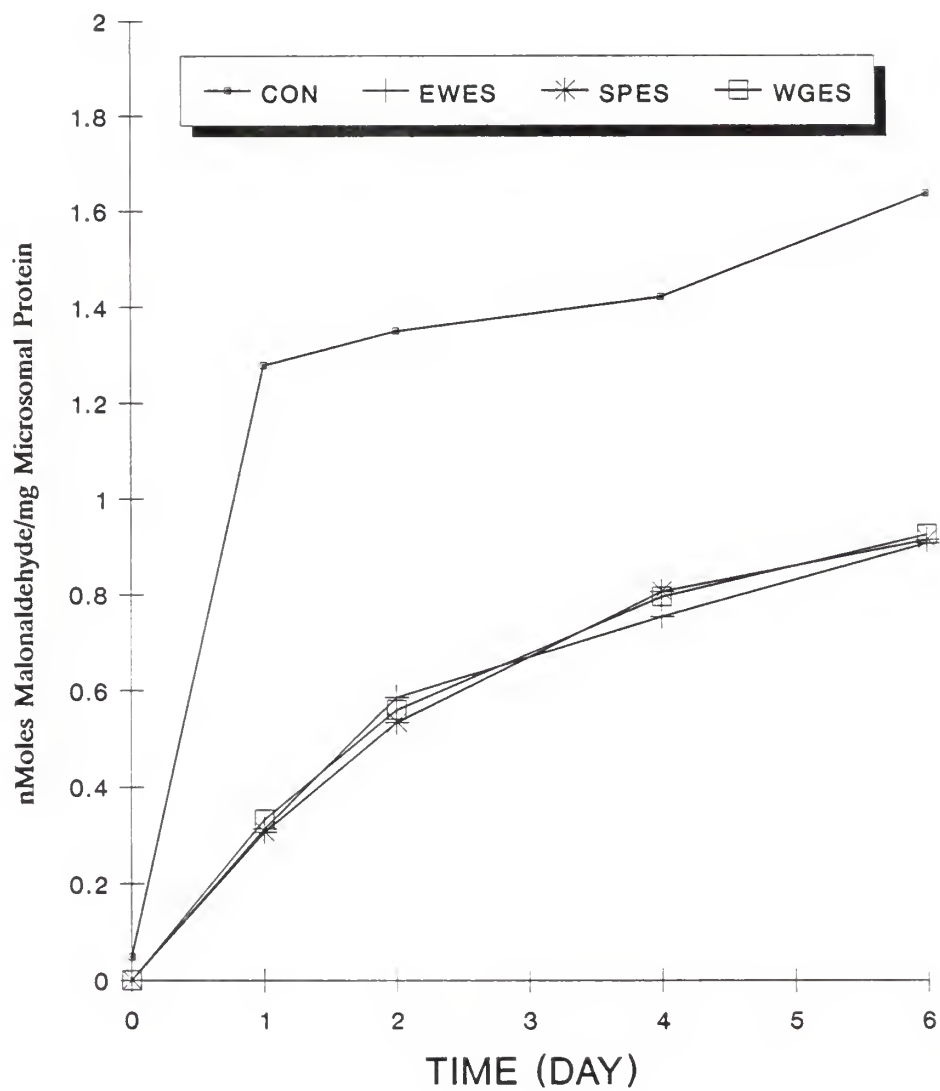
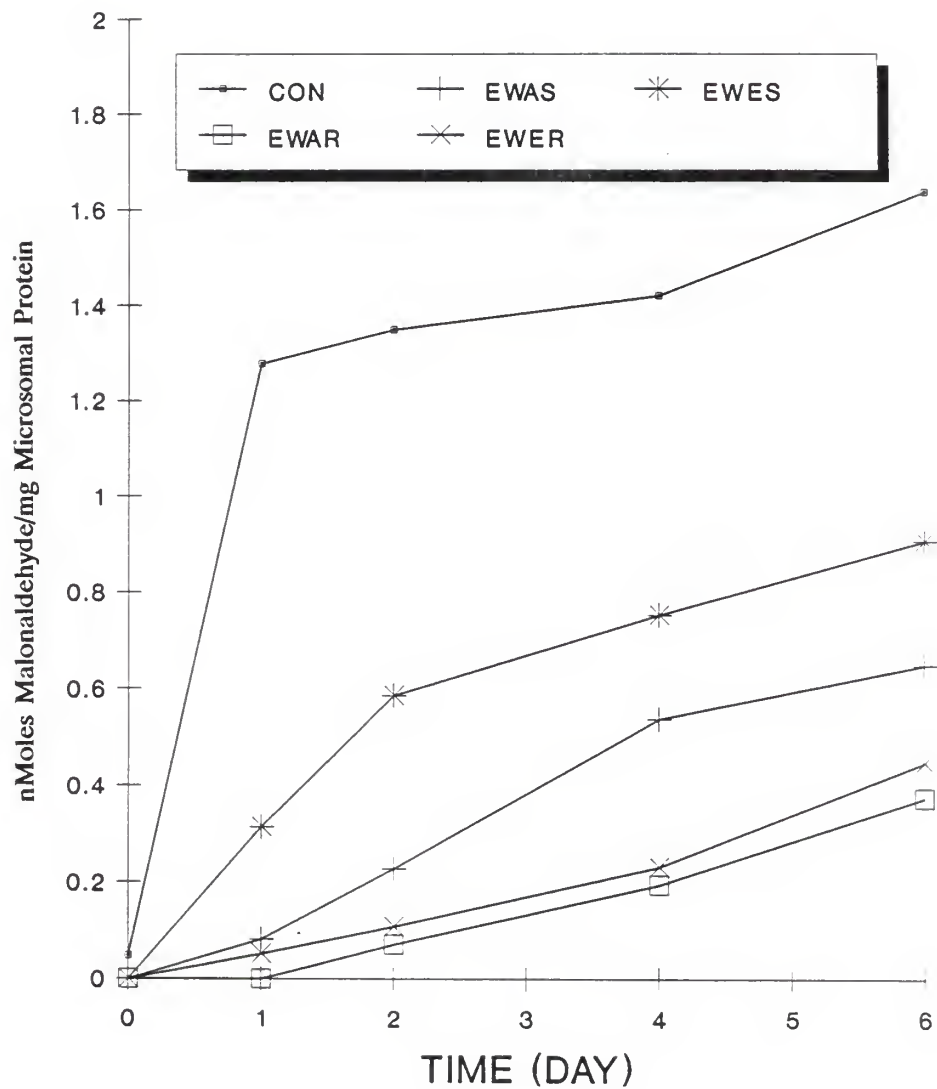


Figure 23. A comparison of the antioxidative activity of MRP formed by spray dried method with those formed by refluxing method of egg white acid or enzymatic hydrolysate on the development of lipid oxidation (as measured by the TBA test) in cooked microsomal membrane lipids from beef tissues stored at 4°C.

CON = control, EWAS = egg white acid hydrolysate, spray dried method, EWES = egg white enzymatic hydrolysate, spray dried method, EWAR = egg white acid hydrolysate, refluxing method, EWER = egg white enzymatic hydrolysate, refluxing method.





lack of large browning polymers such as those formed in the refluxed product appears to result in less effective antioxidant properties. The superior antioxidative efficiency of the refluxed product may be due to the metal chelating characteristics of the large browning polymers.

The results of this study indicate that MRP may have significant antioxidative effect in a cooked meat model system. Further research is needed, however, to investigate their effect in a restructured cooked meat product. These compounds may represent a way of inhibiting the onset of warmed-over flavor in precooked convenience meats. The elimination of warmed-over flavor in these products would greatly enhance their acceptance by consumers and would provide important financial advantages for the meat industry.

## SUMMARY AND CONCLUSIONS

The enzymatic or acid protein hydrolysates of egg white, soy protein or wheat gluten were refluxed or spray dried with D-glucose to form MRP. A model system consisting of the microsomal lipid fraction of semimembranosus beef tissue and activated MetMb-H<sub>2</sub>O<sub>2</sub> was utilized to investigate the relative antioxidative efficiency of the MRP formed in this.

The addition of MetMb-H<sub>2</sub>O<sub>2</sub> caused a 24- fold increase in TBA value for cooked microsomal fraction after 1 day storage. Thus, this rapid rate of lipid oxidation in the cooked meat model system may be due to the formation of a catalytic porphyrin cation radical or to release of non-heme iron from myoglobin.

The results demonstrated that the pattern of antioxidant activity shown by the various MRP was parallel to the extent of protein hydrolysis. Thus, the greater extent of protein hydrolysis resulted in MRP with greater antioxidant activity. This study, supported the theory that those proteins containing a greater proportion of basic amino acids formed the most potent antioxidants. The antioxidative mechanism might be due to the reaction of amine groups with the carbonyls produced during the breakdown of lipid peroxides.

The unfractionated MRP formed by refluxing soy protein enzymatic hydrolysate with glucose was the most effective among the others unfractionated MRP, followed by that of egg white and finally that of wheat gluten in delaying lipid oxidation. Also, the unfractionated MRP for each individual protein source exhibited the strongest antioxidant activity, followed by that of the corresponding low molecular weight

MRP fraction and finally that of the corresponding high molecular weight MRP fraction. Thus, the antioxidant activities of the MRP appear to be primarily due to the low molecular weight fraction.

Although some antioxidative effect was achieved by the acid hydrolysis of egg white alone, the effect was considerably increased by reacting them with glucose. Again, the best antioxidant activity was achieved by unfractionated MRP prepared from acid hydrolysis of egg white followed by corresponding low molecular weight MRP fraction and finally corresponding high molecular weight fraction. The results demonstrate that the MRP of egg white acid hydrolysate was more effective than that of egg white enzymatic hydrolysate.

Among the various protein sources, the strongest antioxidative effect was obtained from soy protein hydrolysate, followed by egg white hydrolysate and wheat hydrolysate. In addition, the formation of effective antioxidative MRP was favored by refluxing method rather than spray-drying method.

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# APPENDIX

TBA values of cooked microsomal lipid fraction of semimembranosus beef tissues catalyzed by MetMb-H<sub>2</sub>O<sub>2</sub> and stored at 4 °C as affected by the addition of MRP prepared from protein hydrolysates and glucose.

TRT	Days of storage at 4 °C				
	0	1	2	4	6
CON	0.05±0.02	1.28±0.05	1.35±0.06	1.42±0.03	1.64±0.01
EWE	0.00±0.00	0.05±0.01	0.11±0.04	0.23±0.01	0.45±0.00
EWEL	0.01±0.01	0.05±0.00	0.10±0.00	0.30±0.01	0.66±0.03
EWEH	0.01±0.01	0.10±0.01	0.33±0.04	1.07±0.02	1.20±0.07
EWES	0.00±0.00	0.31±0.27	0.59±0.23	0.75±0.33	0.91±0.20
EWA	0.00±0.00	0.00±0.00	0.07±0.01	0.19±0.08	0.37±0.00
EWAL	0.03±0.01	0.05±0.01	0.08±0.01	0.15±0.03	0.41±0.06
EWAH	0.03±0.01	0.06±0.01	0.20±0.02	0.29±0.06	0.85±0.02
EWAS	0.00±0.00	0.08±0.10	0.23±0.09	0.54±0.13	0.65±0.12
EWW	0.03±0.01	0.38±0.01	0.70±0.01	0.73±0.00	0.99±0.02
SPE	0.00±0.00	0.00±0.00	0.06±0.01	0.12±0.02	0.19±0.03
SPEL	0.02±0.00	0.06±0.01	0.13±0.01	0.24±0.06	0.48±0.08
SPEH	0.03±0.00	0.12±0.01	0.24±0.02	0.83±0.11	1.17±0.11
SPES	0.00±0.00	0.31±0.27	0.53±0.24	0.81±0.32	0.91±0.24
SPAS	0.00±0.00	0.08±0.02	0.20±0.13	0.44±0.35	0.60±0.27
WGE	0.02±0.01	0.08±0.05	0.20±0.03	0.63±0.00	0.94±0.04
WGEL	0.01±0.01	0.08±0.02	0.50±0.04	0.85±0.05	1.05±0.04
WGEH	0.03±0.00	0.12±0.03	0.75±0.05	0.91±0.03	1.22±0.08
WGES	0.00±0.00	0.33±0.23	0.56±0.25	0.79±0.33	0.92±0.36
WGAS	0.00±0.00	0.15±0.13	0.29±0.17	0.64±0.27	0.77±0.28

Values are expressed in nMole malonaldehyde/mg microsomal protein, ± standard deviation for quadruplet analyses.



INFLUENCE OF MAILLARD REACTION PRODUCTS ON LIPID OXIDATION  
IN A COOKED MEAT MODEL SYSTEM

by

MOHAMMED A. ALFAWAZ

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## ABSTRACT

Egg white, soy protein or wheat gluten was hydrolyzed in this study under acidic conditions or enzymatically. The extent of hydrolysis was monitored spectrophotometrically by reacting free amine groups with TNBS. Maillard reaction products (MRP) were prepared by refluxing the protein hydrolysates with glucose or by spray drying this mixture. A model system consisting of the microsomal lipid fraction of semimembranosus muscle of beef tissue activated by  $\text{H}_2\text{O}_2$ -MetMb was utilized to evaluate the antioxidative effect of the MRP as measured by the TBA assay. The antioxidative effect of the MRP was found to be dependent on the protein source, the method of hydrolysis, the method of forming the MRP and the molecular weight of the MRP fraction. Although some antioxidative effect was exerted by the acid hydrolysate alone, the activity was greatly enhanced by reacting the hydrolysate with glucose. More potent antioxidative MRP were formed from those hydrolysates prepared under acidic conditions as compared to those formed enzymatically. Differences in the antioxidative effect of the various protein sources may be related to differences in the content of basic amino acids. While MRP produced by spray drying demonstrated some antioxidative properties, more potent antioxidants were obtained by refluxing the hydrolysates and glucose. The low molecular weight MRP (MW < 10,000) were observed to have a greater antioxidative effect than the high molecular weight fraction. A possible explanation is that the low molecular weight MRP contain reducing compounds which can quench free radicals. The high molecular weight MRP may inhibit lipid oxidation by chelating prooxidative metals.